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RETENTION AND UTILIZATION OF ORALLY ADMINISTERED IRON

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Although there is a voluminous literature on both iron balances and hypochromic anemia, no systematic study has been made to determine the amount of iron retained by patients with anemia who recover after the administration of massive doses of iron. We wish to present the results of such studies of patients with hypochromic anemia who were given large amounts of iron by mouth.

The effectiveness of iron in the treatment of hypochromic anemia has been repeatedly demonstrated, and failure to obtain the expected therapeutic response is usually due to a complicating infection or to the employment of a suboptimal amount of iron. It has been recognized, however, that the intake of iron is greatly in excess of the amount retained or utilized by the body, and Heath,[1] in confirmation of this view, has shown that only 3.4 per cent of the iron administered orally is utilized in the production of hemoglobin during the period of recovery from anemia. A higher percentage of utilization was observed early in the period of recovery and also when small amounts of iron were administered. His results were obtained by comparing the intake of iron to the amount of iron in the new-formed hemoglobin, assuming that the blood volume was 5 liters in each patient. Heath, Strauss and Castle [2] found that with parenteral administration the percentage of the iron utilized in the formation of hemoglobin varied from 69 to 154, with an average utilization of 96 per cent, so that iron which is given by this route corresponds closely to the amount gained in the circulating

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This work was begun in association with Dr. C. W. Baldridge, who died on Nov. 22, 1934.

1. Heath, C. W.: Oral Administration of Iron in Hypochromic Anemia, Arch. Int. Med. **51**:459 (March) 1933.

2. Heath, C. W.; Strauss, M. B., and Castle, W. B.: Quantitative Aspects of Iron Deficiency in Hypochromic Anemia, J. Clin. Investigation **11**:1293 (Nov.) 1932.

hemoglobin. These results do not account for replacement of the diminished stores of iron in the body, and it seems reasonable to assume that at least a portion of the intake of iron is utilized for this purpose. Hahn and Whipple [3] have shown that the reserve storage of iron in the dog can be exhausted within two or three months by continuous anemia produced by withdrawal of the blood. A similar depletion of the stores of iron is probable in the human being after a chronic loss of blood and possibly in idiopathic hypochromic anemia.

It must be recognized that experiments on the iron balance do not furnish a complete story of the utilization and absorption of iron. This method of study does not distinguish between that portion of the iron which passes unchanged through the gastro-intestinal tract and that which has been utilized and reexcreted into the bowel. Methods for the quantitative determination of the iron content of food and excreta are numerous because none is perfect, and it was this lack of a uniformly acceptable method that led to much difficulty in the determination of the balances of iron when small amounts of this metal were involved. In studies, such as we are presenting, in which the amounts of iron administered are very large, the usual sources of error are of less significance.

METHODS

Iron and ammonium citrates was selected for use in this investigation because of its known potency in the treatment of these types of anemia. It was administered as an aqueous solution of the brown scales, 15 grains (1 Gm.) three times daily, equivalent to approximately 500 mg. of metallic iron per day. One patient (case 5) received a solution of the green scales, which contained a somewhat smaller amount of iron. No distinction was made as to the presence or absence of hydrochloric acid in the gastric contents, since we were concerned primarily with the amount of iron retained and utilized by the body.

Loss of menstrual blood was encountered in five of the nine women during the studies. The amount of iron lost in the menstrual flow was regarded in the calculations as eliminated iron, so that the positive balances represent the iron retained in spite of this loss.

The subjects selected for these metabolic studies were patients with a microcytic hypochromic type of anemia, and a brief summary of the clinical hematologic and laboratory data is given in tables 1 and 2. Balance periods were of six days' duration, and in each case the control period was preceded by a three day period of adjustment on the balance diet before observations were begun. The balance periods ran continuously except in case 1, in which there was an interval of fifty days between the fifth and the sixth period and in case 2, in which there was an interval of twenty-four days between the third and fourth period. Carmine was used to mark the stools at the beginning and at the end of each balance period.

Three standard diets were adjusted to contain 1 Gm. protein per kilogram of body weight and a caloric intake of about 150 per cent of the basal requirements.

3. Hahn, P. F., and Whipple, G. H.: Iron Metabolism: Its Absorption, Storage and Utilization in Experimental Anemia, Am. J. M. Sc. **191**:24 (Jan.) 1936.

The ratio of acid to base in the diet was approximately 1:2.3. The compositions of the three standard diets, as found by analysis and by calculation from the tables of Rose,[4] were almost identical, so that in computing the iron balances the iron in the food was determined by calculation from these tables rather than by analysis

TABLE 1.—*Data for Patients with Anemia*

Patient	Age	Sex	Diagnosis	Clinical Features
1	26	F	Hypochromic anemia; menorrhagia	Repeated severe uterine hemorrhages for 6 years; hemorrhage from temporal artery after an injury; hypochlorhydria
2	47	M	Hypochromic anemia; marginal ulcer	Peptic ulcer for 15 years with several severe hemorrhages; gastro-enterostomy performed but bleeding persisted; koilonychia
3	46	F	Hypochromic anemia; menorrhagia	Menorrhagia for 4 years, relieved by application of radium 7 months before balance studies; hypochlorhydria
4	49	F	Hypochromic anemia; menorrhagia	Climacteric bleeding; dilation and curettage of uterus showed normal endometrium
5	37	F	Hypochromic anemia; atrophic arthritis	No abnormal loss of blood; dysphagia; hypochlorhydria; atrophy of tongue; anemia more severe than in ordinary arthritis and responded to iron
6	38	F	Hypochromic anemia; steatorrhea	No abnormal loss of blood; frequent, profuse, bulky stools since age of 15; sore mouth and atrophic tongue; koilonychia; dysphagia; hypochlorhydria
7	39	F	Hypochromic anemia of pregnancy	Eight pregnancies; no abnormal loss of blood; hemoglobin, 55 per cent prior to last delivery
8	32	F	Hypochromic anemia (idiopathic)	No history of abnormal loss of blood; adequate diet; atrophic tongue; koilonychia; splenomegaly; hypochlorhydria
9	44	F	Hypochromic anemia (idiopathic)	Dysphagia; no abnormal loss of blood; atrophic tongue; koilonychia; latent syphilis; achlorhydria
10	35	F	Hypochromic anemia (idiopathic)	Dysphagia; atrophic tongue; no abnormal loss of blood; achlorhydria

TABLE 2.—*Data for Patients with Anemia* *

Patient	Hemoglobin, %	Hematocrit, %	Erythrocytes, %	Color Index	Volume Index	Saturation Index	Gastric Acidity	Basal Metabolic Rate
1.....	30	48	69	0.43	0.70	0.62	Low	— 8
2.....	10	15	29	0.34	0 51	0.66	0	— 1
3.....	54	69	109	0.50	0.60	0.78	0	— 4
4.....	75	72	95	0.78	0.75	1.04	Normal	± 0
5.....	40	68	90	0.44	0.76	0.60	Low	— 6
6.....	30	60	101	0.29	0.60	0.65	Low	—11
7.....	39	61	102	0.38	0.59	0.64	0	+ 7
8.....	42	68	84	0.50	0.80	0.62	Normal	+10
9.....	44	65	81	0.54	0.84	0.67	0	— 6
10.....	32	52	76	0.42	0.68	0.62	0	—20

* The percentages were calculated from the normal values of Osgood (A Textbook of Laboratory Diagnosis, ed. 2), Philadelphia, P. Blakiston's Son & Co., 1935, p. 420.

of the food. These diets were alternated during the six day periods. The food did not come in contact with metal during its preparation, except occasionally with aluminum. The drinking water, which contained less than 0.1 mg. of iron

4. Rose, Mary S.: Laboratory Handbook for Dietetics, ed. 3, New York, The Macmillan Company, 1929.

per liter, was not considered in calculating the balances. The iron content of the iron and ammonium citrates and other medications was determined by analysis. All excreta were carefully collected and stored in glass or porcelain-lined containers, and all possible precautions were taken to prevent contamination.

Nitrogen was determined by the Kjeldahl method and phosphorus by the method of Fiske and Subbarow.[5] All determinations of iron were made in triplicate by the method of Reis and Chakmakjian,[6] and the accuracy of this method was repeatedly verified by the recovery of added iron from representative specimens.

The hemoglobin content was determined by the Newcomer method[7] and the hematocrit values by the method of Van Allen.[8] Erythrocyte, leukocyte and reticulocyte counts and hemoglobin and hematocrit determinations were made daily or on alternate days. Differential leukocyte counts were made during each balance period.

RESULTS

The control periods in these cases are open to all the criticisms that obtain in iron balances under physiologic conditions. The diet contained from 9 to 12 mg. of iron per day, and on this approximately normal intake six patients were in negative and four in positive iron balance. The patient who presented the lowest negative balance (patient 2) was losing a small amount of blood continuously from a gastrojejunal ulcer, which would tend to lower the iron balance and to account partially for this extremely low result. Patient 7 received 168.6 mg. of iron by mistake on the last day of the control period and during the six day period retained 106.8 mg. She received the same diet which resulted in a negative or a slightly positive iron balance in the other patients, so that most, if not all, of the retained iron came from the single dose of iron and ammonium citrates.

Retention.—With the administration of iron and ammonium citrates these patients retained a suprisingly large amount of iron. The complete results of the studies are given in table 3 and a summary of the retention of iron by periods in table 4. The first patient retained 6,760.5 mg. of iron in forty-eight days, and patient 9 retained 8,854.4 mg. in twenty-four days. The greatest retention occurred in the first and second periods of the administration of iron, with a decreased retention in the subsequent ones. In each of the second, third and fourth periods there was one patient in negative iron balance, but no patient presented this feature in more than one period. Patient 1, whom we

5. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**:375 (Dec.) 1925.

6. Reis, F., and Chakmakjian, H. H.: Colorimetric Method for Quantitative Determination of Iron in Blood in the Form of Dispersed Prussian Blue, J. Biol. Chem. **92**:59 (June) 1931.

7. Newcomer, H. S.: A New Optical Instrument for the Determination of Hemoglobin, J. Biol. Chem. **55**:569 (April) 1923.

8. Van Allen, C. M.: An Hematocrit Method, J. Lab. & Clin. Med. **10**:1027 (Sept.) 1925.

TABLE 3.—*Summary of the Data*

TABLE 3.—*Summary of the Data*

Patient	Period	Weight, Kg.	Nitrogen, Mg.			Phosphorus, Mg.			Iron, Mg.			Erythrocytes, Millions	Hemoglobin, Gm.
			Intake	Excretion	Balance	Ingestion	Excretion	Balance	Ingestion	Excretion	Balance		
1	1	52.5	10.90	12.28	−1.38	1.53	1.51	−0.02	10.0	19.9	− 9.9	3.44	4.34
	2	52.4	11.52	10.87	+0.65	1.58	1.31	+0.27	516.1	477.9	+ 38.2	3.54	5.01
	3	52.3	11.93	12.74	−0.81	1.61	1.28	+0.33	516.1	252.3	+263.8	3.84	6.38
	4	52.7	11.47	10.59	+0.88	1.58	1.02	+0.56	516.1	559.6	− 43.5	4.34	6.72
	5	53.1	11.49	10.35	+1.14	1.57	1.32	+0.25	516.1	481.7	+ 34.4	4.52	8.62
	6*	53.1	11.49	12.91	−1.42	1.57	1.44	−0.13	516.1	446.9	+ 69.2	4.22	8.56
	7	55.0	11.32	12.55	−1.03	1.58	1.19	+0.39	516.1	211.8	+304.3	4.30	10.55
	8	55.2	11.61	10.79	+0.82	1.60	1.30	+0.30	516.2	251.7	+264.5	3.71	8.98
	9	56.2	11.62	8.63	+2.99	1.61	1.41	+0.20	516.2	320.2	+196.0	3.63	9.34
2	1†	66.3	7.40	7.61	−0.21	1.05	0.99	+0.06	7.2	24.9	− 17.7	1.44	1.55
	2	68.6	7.08	7.89	−0.81	1.08	0.62	+0.46	513.5	419.9	+ 93.6	1.70	1.60
	3	69.5	9.05	7.04	+2.01	1.28	0.97	+0.31	514.7	351.7	+163.0	1.78	1.83
	4‡	68.1	18.69	12.62	+6.07	1.81	1.19	+0.62	522.0	184.2	+337.8	2.88	3.92
	5	68.8	18.58	12.52	+6.06	1.78	1.14	+0.64	522.6	361.0	+161.6	3.13	4.27
3	1	69.0	9.22	8.89	+0.33	1.10	1.18	−0.08	9.1	19.9	− 10.8	5.45	7.68
	2	69.6	10.20	9.05	+1.15	1.18	1.17	+0.01	515.8	290.5	+225.3	5.40	7.79
	3	69.5	10.20	8.88	+1.32	1.18	1.16	+0.02	515.8	290.6	+225.2	5.75	8.20
4	1	76.6	9.98	9.69	+0.29	1.19	1.12	+0.07	10.1	12.2	− 2.1	4.84	10.33
	2	76.8	10.28	8.94	+1.34	1.19	0.85	+0.34	515.9	153.8	+362.1	4.91	10.40
5	1	56.9	10.22	8.42	+1.80	1.49	1.03	+0.46	12.6	9.7	+ 2.9	4.51	5.68
	2	57.2	11.14	8.29	+2.85	1.53	1.63	−0.10	478.1	187.4	+290.7	4.25	5.76
	3	60.0	11.18	9.62	+1.56	1.57	1.55	+0.02	477.8	317.1	+160.7	4.76	6.33
	4	59.5	10.20	8.50	+1.70	1.44	1.38	+0.06	476.7	219.7	+257.0	5.23	7.09
	5	59.2	10.18	9.91	+0.27	1.43	1.52	−0.09	476.8	581.2	−104.4	4.92	7.67
6	1	52.0	9.89	9.92	−0.03	1.18	1.10	+0.08	38.1	18.6	+ 19.5	5.05	5.56
	2	52.0	10.17	9.34	+0.83	1.18	1.07	+0.11	515.9	357.2	+158.7	5.11	6.22
	3	52.2	9.32	9.10	+0.22	1.10	1.00	+0.11	514.7	520.5	− 5.8	5.31	7.16
	4	53.7	9.96	9.65	+0.31	1.16	1.21	−0.05	515.4	450.6	+ 64.8	5.13	7.28
7	1	61.4	10.06	8.23	+1.83	1.16	1.14	+0.02	10.0	21.0	− 11.0	3.86	7.65
	2	62.1	10.10	9.41	+0.69	1.15	1.14	+0.01	515.7	334.3	+181.4	3.92	7.64
8	1	45.0	7.38	7.78	−0.40	1.02	0.99	+0.03	12.7	12.5	+ 0.2	4.26	6.01
	2	44.1	9.27	9.04	+0.23	1.29	1.34	−0.05	519.2	227.3	+241.9	4.65	7.59
	3	48.8	9.54	8.41	+1.13	1.35	1.38	−0.03	519.4	227.4	+292.0	4.49	7.72
9	1	80.9	9.79	11.31	−1.52	1.44	1.51	−0.07	11.9	11.8	+ 0.1	4.45	6.17
	2	80.3	10.95	9.37	+1.58	1.44	1.21	+0.23	517.3	185.7	+331.6	3.75	5.91
	3	81.1	10.18	8.12	+2.06	1.44	1.03	+0.41	516.9	110.5	+406.4	4.21	5.83
	4	80.3	10.26	6.72	+3.54	1.47	1.03	+0.44	517.2	165.6	+351.6	4.09	5.97
	5	79.7	10.25	8.04	+2.21	1.67	1.03	+0.64	517.2	130.9	+386.3	4.06	6.53
10	1	51.7	9.01	8.25	+0.76	1.08	0.99	+0.09	9.7	17.5	− 7.8	3.80	4.62
	2	52.0	10.19	8.58	+1.61	1.19	0.80	+0.39	516.3	260.4	+255.9	4.02	4.72
	3	51.9	10.07	9.15	+0.92	1.18	1.27	−0.09	516.2	215.8	+300.4	3.82	5.30
	4	51.5	10.19	9.33	+0.86	1.19	1.05	+0.14	516.3	267.3	+248.9	4.21	6.29
	5	51.8	10.20	9.36	+0.84	1.19	1.24	−0.05	516.7	277.2	+239.5	4.90	7.17

* There was a fifty day interval between periods 6 and 7.
† There was a three day control period. All other periods were six days long.
‡ There was a twenty-four day interval between periods 3 and 4.

were able to follow by means of metabolic studies for a total of nine six day periods, presented some interesting features. An interval of fifty days elapsed between the fifth and the sixth balance period, during which time the patient took iron and ammonium citrates, 45 grains (3 Gm.) daily, except for a lapse of three days. In spite of this prolonged period of administration of iron, she still showed a markedly positive balance during the subsequent metabolic studies, and during the period of observation alone she retained 6.76 Gm. of iron. How long these large amounts of iron are retained by the body we are unable to say, but the continued storage at the end of ninety-eight days of the administration of iron shows that it is more than a transient affair.

The experience with patient 6, who received iron on the last day of the control period, suggests that absorption and retention are rapid in spite of the delay that occurs in the response of the hemoglobin. It has

TABLE 4.—*Average Daily Iron Balances (Mg.) by Periods*

Patient	Control Period	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8	Total Iron Balance
1	— 9.9	+ 38.2	+263.8	— 43.5	+ 34.4	+69.2	+304.3	+264.5	+196	6,760.5
2	—17.7	+ 93.6	+163.0	+337.8	+161.6	.....	.....	.....	.....	4,535.5
3	—10.8	+225.3	+225.2	.....	.....	.....	.....	.....	.....	2,702.7
4	— 2.1	+362.1	.....	.....	.....	.....	.....	.....	.....	2,173.0
5	+ 2.9	+290.7	+160.7	+257.0	—104.4	.....	.....	.....	.....	3,624.4
6	+19.5	+158.7	— 5.8	+ 64.8	.....	.....	.....	.....	.....	1,305.0
7	—11.0	+181.4	.....	.....	.....	.....	.....	.....	.....	1,088.2
8	+ 0.2	+241.9	+292.0	.....	.....	.....	.....	.....	.....	3,203.2
9	+ 0.1	+331.6	+406.4	+351.6	+386.3	.....	.....	.....	.....	8,854.4
10	— 7.8	+255.9	+300.4	+248 9	+239.5	.....	.....	.....	.....	6,268.4
Average	— 4.8*	+217.9	+225.7	+202.8	+143.5	.....	.....	.....	.....	.....

* Exclusive of cases 2 and 6 (see text).

been stated by Lintzel [9] that the retention of such large amounts of iron is pathologic and occurs only because the duodenal mucosa has been damaged; but if this were true, surely the absorption of 106.8 mg. from a single dose of 168.6 mg. of iron could not have occurred.

Utilization.—The anemia in all cases responded well to the administration of iron and ammonium citrates, and there was a gradual, although irregular, increase in the hemoglobin content of the blood. The usual reticulocyte response was obtained. Heath [1] has shown that the normal response of the hemoglobin to the administration of iron when the initial hemoglobin level is below 7.8 Gm. per hundred cubic centimeters is an increase of 0.16 Gm. per day. An increase of this amount was obtained at times in certain patients, but in no instance did the average for an entire experimental period equal this figure (column

9. Lintzel, W.: Neuere Ergebnisse der Erforschung des Eisenstoffwechsels, Ergebn. d. Physiol. **31**:844, 1931.

5, table 6). The amount of iron which was administred (iron and ammonium citrate, 15 grains [1 Gm.] three times a day, giving approximately 500 mg. of metallic iron per day) was only half the dose regularly employed in clinical work and may be considered as suboptional if judged by the results obtained by Heath. Table 5 gives the average daily response of the hemoglobin for all patients, together with the average daily retention of iron. In the fourth period the response of the hemoglobin was the greatest, and it closely approximated the expected increase. It is to be noted that there was no correlation between the amount of iron retained and the increase in the hemoglobin content. A summary of the iron balances, the increase in the hemoglobin

TABLE 5.—*Average Daily Iron Balance and Increase in Hemoglobin Content*

	Control Period	Period 1	Period 2	Period 3	Period 4
Number of patients	8	10	9	6	5
Average daily iron balance, mg.	−4.8	+217.9	+225.7	+202.8	+143.5
Average daily increase in hemoglobin value, Gm. per 100 Cc.		0.058	0.111	0.056	0.157

TABLE 6.—*Data on Retained Iron Utilized as Hemoglobin*

Patients	Average Daily Iron Balance, Mg.	Total Intake of Iron, Gm.	Total Iron Retained, Gm.	Original Hemoglobin, Gm. per 100 Cc.	Average Daily Hemoglobin, Gain Gm. per 100 Cc.	Total Hemoglobin Gain, Gm.	Iron: Retained, %	Iron: Used as Hemoglobin, %	Retained Iron Utilized as Hemoglobin, %
1	140.8	24.773	6.760	4.34	0.1130	272.00	27.8	3.20	11.80
2	189.0	12.436	4.535	1.55	0.0416	50.60	36.4	1.20	3.30
3	225.2	6.189	2.702	7.68	0.1170	70.50	43.6	3.41	7.82
5	151.0	11.456	3.624	5.68	0.0920	111.00	31.6	2.90	9.20
6	72.6	9.276	1.305	5.56	0.0950	85.80	14.0	2.77	19.70
8	266.9	6.231	3.208	6.01	0.0560	33.75	51.4	1.62	3.16
9	368.9	12.411	8.854	6.17	0.0870	45.25	71.3	1.09	1.53
10	261.2	12.392	6.268	4.62	0.1170	141.50	50.5	3.42	6.77

content and the utilization of iron is given in table 6. Since patients 4 and 7 were under observation for only one six day period of administration of iron, a period too short to be of significance as far as the formation of hemoglobin is concerned, they were omitted from this table. In calculating the iron utilized in the formation of hemoglobin we have assumed, as did Heath,[1] that the blood volume in each instance was 5 liters. Although this is only an approximation, it is sufficiently accurate so that the percentage of utilization based on this figure is of significance. It is unfortunate that determinations of the blood volume were not made for all the patients. The total increase of the hemoglobin content in 5 liters of blood was determined from the hemoglobin gained per hundred cubic centimeters. The iron content of this hemoglobin

was designated as the iron utilized in the formation of hemoglobin. From this was calculated the percentage of the administered iron and of the retained iron which was so utilized.

The percentage of iron retained by the body varied from 14 to 71.3 per cent of that administered by mouth. This was highest in the cases of idiopathic hypochromic anemia (cases 8 to 10), but the difference between the lowest retention in this group and the highest retention in the others was so slight that its significance is doubtful. The average retention for the entire group was 32.6 per cent.

The percentage of administered iron that was utilized in the formation of hemoglobin varied from 1.2 to 3.42, with an average of 1.96 per cent. This is definitely lower than the average of 3.4 per cent found by Heath. The percentage of retained iron that was utilized as hemoglobin varied from 1.53 to 19.7, with an average of 6.32 per cent. This indicates that by far the largest part of the iron which is retained within the body is deposited in some form other than hemoglobin in the blood.

There was no correlation between the original hemoglobin level and the amount of iron retained or the percentage utilized, and the type of anemia had no bearing on these results.

The average daily nitrogen balance for all patients during the control periods was plus 0.18 Gm., and with the administration of iron it increased to plus 1.32 Gm. per day. The phosphorus balance increased from plus 0.07 Gm. in the control periods to plus 0.19 Gm. during the administration of iron. This is not in accord with the results of animal experimentation, which suggest that the daily administration of iron leads to a decrease in the phosphorus content of the blood.[10]

The total leukocyte and differential counts showed no significant changes during iron therapy.

COMMENT

A summary of the present concept of iron metabolism is as follows: Absorption of iron administered by mouth takes place throughout the entire gastro-intestinal tract but occurs mainly in the duodenum and in the upper portion of the small intestine.[11] The iron is eliminated almost entirely through the gastro-intestinal tract, chiefly through the colon. The processes of elimination and absorption are reversible and

10. Brock, J. F., and Diamond, L. K.: Rickets in Rats by Iron Feeding, J. Pediat. **4**:442 (April) 1934.

11. (*a*) Robscheit-Robbins, F. S.: The Regeneration of Hemoglobin and Erythrocytes, Physiol. Rev. **9**:666 (Oct.) 1929. (*b*) M'Gowan, J. P.: The Absorption and Excretion of Iron by the Intestines and the Nutritional and Therapeutic Value of Its Salts, Edinburgh M. J. **37**:85 (Feb.) 1930.

may occur at the same location.[12] Small amounts are eliminated in the urine and bile, but even parenterally administered iron is excreted almost entirely through the intestinal tract.[9] The absorbed iron is transmitted mainly, if not entirely, by the blood stream, although the form in which it is transported is unknown.[13] It has been shown, however, that the oral administration of iron in large doses does not appreciably increase the iron content of the blood plasma outside the portal circulation.[14] It may be accepted from animal experimentation that the liver is the principal storehouse for iron.[15] Smaller amounts are stored in other parts of the reticulo-endothelial system, and aside from the liver, the spleen is the most important storage depot. The iron content of the liver may be increased by the administration of iron to eight times its normal value and that of the spleen to four times its normal value.[11a] There is no decrease in the iron content of muscle (muscle hemoglobin and parenchymal iron) with long continued anemia, nor is this value increased with the administration of large amounts of the metal.[8]

Our observations have established the fact that large amounts of iron are retained in the body as a result of the oral administration of iron and ammonium citrates, and from the aforementioned results of animal experimentation it seems logical to conclude that this iron is retained primarily in the liver and spleen. If our present ideas concerning the total iron content of the body are correct (blood hemoglobin, muscle hemoglobin and parenchymal or respiratory iron of the cells), it must be admitted that all the 6.76 Gm. retained in case 1 and the 8.85 Gm. retained in case 9 could not be put to a useful purpose immediately when such a small percentage was utilized in the formation of hemoglobin. The storage capacity of the liver and its efficiency in removing iron from the portal circulation must be great, since the amount of iron in the plasma of the general circulation is not elevated appreciably. When one realizes the amount of iron retained and its probable storage in the liver, it must cause some concern over the possibility of the development of pigmentary cirrhosis of this organ similar to that which occurs in hemochromatosis. Although we are not aware that any case of this type has been reported, the possibility seems real in those cases in which iron is administered over a long period.

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12. Verzar, F., in Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1929, vol. 4, p. 72. M'Gowan.[11b]

13. Lintzel.[9] Robscheit-Robbins.[11a]

14. Marlow, A., and Taylor, F. H. L.: Constancy of Iron in the Blood Plasma and Urine in Health and in Anemia, Arch. Int. Med. **53**:551 (April) 1934.

15. Polson, C. J.: The Storage of Iron Following Its Oral and Subcutaneous Administration, Quart. J. Med. **23**:77 (Oct.) 1929. Lintzel.[9] Robscheit-Robbins.[11a]

Lintzel [9] stated the belief that the retention of such large amounts of iron is pathologic. He said that the body finds a rate of excretion of iron which agrees with the intake, so that it is soon in balance, and when 100 or 200 mg. of iron is given its excretion is prompt. Our results indicate that large amounts are retained by the body, and the retention of 63 per cent of a single dose of iron in case 6 does not bear out Lintzel's supposition that the iron first damages the mucosa before being absorbed.

The intermediary steps in iron metabolism are unknown, but it has been suggested that idiopathic hypochromic anemia may be due to a faulty internal metabolism of iron. This hypothesis is not borne out by these experiments, since the patients with an idiopathic form of anemia (patients 8 to 10) retained iron as well as, if not better than, the others and also showed a percentage utilization in hemoglobin formation which was equal to the utilization by those with chronic hemorrhage.

It must be admitted that our conclusions as to the utilization of iron in the formation of hemoglobin are approximations, since determinations of the blood volume are lacking. The results are somewhat lower than those obtained by Heath,[1] even though we were giving a smaller amount of iron, which should have resulted in a higher percentage of utilization. More significant is the percentage of the retained iron that goes to form hemoglobin, indicating that a large part of the iron that is retained is held in storage rather than being utilized immediately. This is in keeping with the experimental work of Whipple,[16] who found that the optimum dose of iron for dogs rendered anemic by repeated loss of blood exceeded by threefold the iron removed in the hemoglobin. He concluded that the iron had some effect on the body aside from the replacement of hemoglobin.

It has been shown by Heath [2] that 96 per cent of the iron administered to patients parenterally is utilized in the formation of hemoglobin, and similar results were obtained by Whipple [17] in animal experiments. Whipple reasoned by analogy that, since iron administered intravenously is utilized quantitatively in the formation of hemoglobin, the amount of iron absorbed from the gastro-intestinal tract after oral administration may be estimated from the amount of new-formed hemoglobin. This assumption is not verified by our experiments on human beings, which showed that only a small percentage of the retained iron is so utilized.

16. Whipple, G. H., and Robscheit-Robbins, F. S.: Blood Regeneration in Severe Anemia: Optimum Iron Therapy and Salt Effect, Am. J. Physiol. **92**:362 (March) 1930.

17. Whipple, G. H., and Robscheit-Robbins, F. S.: Iron and Its Utilization in Experimental Anemia, Am. J. M. Sc. **191**:11 (Jan.) 1936.

Where the remainder of this is stored can only be surmised, but it seems probable that it remains in the liver, the spleen and the reticulo-endothelial system.

SUMMARY

With the oral administration of iron and ammonium citrates to patients with hypochromic anemia large amounts of iron are retained by the body.

An average of 32.6 per cent of the iron administered was retained by the body.

Approximately 1.96 per cent of the iron administered was utilized in the formation of hemoglobin.

There was no correlation between the amount of iron retained and the increase in the hemoglobin content.

CHRONIC NEPHRITIS IN RATS FED HIGH PROTEIN DIETS

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The metabolism of carbohydrate and fat results in almost complete oxidation or storage of these substances in the body. In contrast, the metabolism of protein leads to the formation of several end-products that are eliminated by the kidney, and the excretion of these products entails work by the kidney. The possibility that this work or some specific product or products may eventually lead to pathologic changes in this organ has been the incentive for much study. The results obtained by feeding animals, chiefly rabbits and rats, diets high in protein have been conflicting. Many of the early investigations are open to the criticism that the necessary food accessories were missing from the diet. The use of rabbits for this type of study is questionable, unless the effects of vegetable proteins are being determined. That in these animals spontaneous nephritis is prone to develop is, in any case, disturbing. The nutritive requirements of the rat are better understood, and it is known to thrive on diets containing animal proteins. But even with rats, the results obtained by different investigators have been at variance. Possibly differences in diets which appear to be slight may account for some of these discordances. The best positive results have been obtained by Newburgh and Curtis,[1] who found that diets containing large quantities of beef liver produce severe renal damage. Diets containing large quantities of beef muscle also were found to be injurious, while casein proved to be relatively harmless. No useful purpose would be gained by considering at length the many other experiments reported in the

From the Biochemical Laboratory and the Hegeman Memorial Laboratory of the Metropolitan Life Insurance Company.

1. Newburgh, L. H., and Curtis, A. C.: Production of Renal Injury in the White Rat by the Protein of the Diet, Arch. Int. Med. **42**:801 (Dec.) 1928.

literature. The reader who is interested in the details is referred to the excellent reviews by Bischoff [2] and Mitchell.[3]

The conflicting results just mentioned led us to undertake the experiments recorded in this paper, in the hope that by giving special attention to the care of the rats and to the composition of their diets we might obtain more uniform and reliable results, which would be useful in explaining certain phenomena of nephritis. A preliminary report of some of our first experiments appeared in 1931.[4]

PLAN OF EXPERIMENTS AND METHODS

The albino rats used were bred in our laboratory from stock obtained originally from the Wistar Institute. Within a few days after weaning they were placed on the experimental diets. They were kept in individual cages of the Yale type, which were changed and sterilized twice weekly. A fresh blotter for the bottom of each cage pan was supplied daily, except Sundays. Food and water were always available, while supplements of carrots and lettuce were fed twice weekly. Accurate records were kept of the body weight and consumption of food. Since February 1933 the animals have been housed under favorable surroundings in an air-conditioned room maintained constantly at a temperature of 23 or 24 C.

Specimens of urine were collected by placing the rats overnight in metabolism cages without food. Samples were obtained monthly, beginning one month after starting the experiment. The entire sediment from each specimen was examined for all formed elements. Albumin was estimated quantitatively by the sulfosalicylic acid method.

The rats were allowed to live until it was evident that death would soon occur. They were then anesthetized with sodium amytal, and samples of blood were removed from the heart for chemical analysis. The urea, nonprotein nitrogen, albumin, globulin and cholesterol contents of the blood and plasma were determined by conventional methods. The kidneys and other organs, after their gross appearance had been noted, were removed, measured, weighed and transferred to a dilute solution of formaldehyde U. S. P. (1:10) or to Zenker's solution for subsequent histologic examination.

The term nephrectomy is used in this paper to mean the removal of the right kidney. This operation was performed with the animal under anesthesia induced by sodium amytal. That the young rats stood this operation well is indicated by their desire to eat soon after the effect of the anesthetic disappeared and by an operative mortality of less than 1 per cent.

Since the beginning of this study in 1929 the experimental work has all been carried on in the biochemical laboratory of the Metropolitan Life Insurance Company in New York city; the histologic examinations have been made at the Hegeman Memorial Laboratory in Mt. McGregor, N. Y. Throughout the study the

2. Bischoff, F.: The Influence of Diet on Renal and Blood Vessel Changes, J. Nutrition **5**:431, 1932.

3. Mitchell, H. H.: The Physiological Effects of Protein, J. Nutrition **1**:271, 1929.

4. Blatherwick, N. R.; Medlar, E. M.; Connolly, J. M., and Bradshaw, P. J.: Nephritis in Unilaterally Nephrectomized White Rats Living upon High Protein Diets, J. Biol. Chem. **92**:lxxxiv, 1931.

only data submitted to the pathologist were the number of the rat and the number of the diet on which it was fed. The results of his histologic examinations were not revealed to the workers in the city of New York until late in the course of the work, after the organs of more than 800 rats had been examined. The total number of rats used exceeded 1,000. The data here presented represent the most significant findings of our study.

PATHOLOGIC EFFECTS OBTAINED WITH DIFFERENT DIETS

A good criterion of whether the diet can produce chronic nephritis is evidence of histologic change in the renal structure. In the pathologic analyses to be presented here the following terms are used to indicate the extent of renal involvement:

Normal: There is no evidence of abnormality in the section of kidney.

1 plus: The glomeruli and the tubular epithelium appear normal. There may be a few small collections of lymphocytes or an occasional tubular cast. Such kidneys do not represent a true condition of nephritis, but they are not entirely normal.

2 plus: Sclerosing glomeruli, abnormal tubular epithelium, tubular casts and collections of lymphocytes may all be present. Less than one fourth of the section is abnormal.

3 plus: The same as 2 plus except that more than one fourth and not over two thirds of the tissue is involved. Fibrosis is usually present.

4 plus: The same as 2 plus except that two thirds or more of the tissue is involved. Fibrosis is always present and may be marked.

In most instances histologic examination was made of the heart, liver, spleen, adrenal glands and aorta in addition to the kidney of each animal. Abnormal conditions were frequently noted in organs other than the kidney, especially calcification of the aorta, but at present it is not possible to correlate all these changes directly and consistently with the renal pathologic picture. Consequently, we have presented in the accompanying tables the data relative to the kidney and aorta only.

Table 1 gives a general summary of the data for 418 rats. The rats are divided as to sex and as to whether nephrectomy had been performed. It will be noted that in the nephrectomized rats nephritis developed much more frequently than in the animals with both kidneys. It is evident, also, that severe nephritis occurred more frequently in males than in females. This difference between the sexes was especially marked in the non-nephrectomized animals. Death occurred earlier in the male rats, the shortest period of survival being among the nephrectomized males.

The combined data for all the diets fed are given in table 1, and the data for each individual diet are analyzed in tables 2 to 7. In table 2 are the results for the rats fed stock diet 2, which has the following composition:

Stock Diet 2	Percentage
Wheat	55
Milk	25
Beef muscle	12
Yeast A	5
Sodium chloride	2
Calcium carbonate	1

The milk and yeast in this and subsequent diets are dry. (Several brands of yeast were employed in the various diets, and information in this regard will be furnished on request.) Lean beef muscle is carefully freed of fat and connective tissue, passed through a chopper, dried at a moderate temperature and then pulverized.

TABLE 1.—*Summary of the Data for All the Rats*

				Nephritis						Aorta		
Sex	Number of Rats	Nephrectomy	Average Length of Life, Days	0	1+	2+	3+	4+	3+ or 4+, %	Number Examined	Number Calcified	Calcified, %
M	65	Yes	430	2	2	5	9	47	86.1	24	10	41.7
M	122	No	515	53	26	17	16	10	21.3	119	4	3.3
F	92	Yes	512	9	7	11	25	40	70.6	42	7	16.6
F	139	No	516	74	40	16	6	3	6.4	123	0	0.0
Total or average	418		504	138	75	49	56	100	37.3	308	21	6.8

TABLE 2.—*Summary of the Data for the Rats Fed Stock Diet 2*

				Nephritis						Aorta		
Sex	Number of Rats	Nephrectomy	Average Length of Life, Days	0	1+	2+	3+	4+	3+ or 4+, %	Number Examined	Number Calcified	Calcified, %
M	3	Yes	531	2	1	..	..	..	..	..	..	...
M	16	No	574	10	6	..	..	..	..	9	..	...
F	5	Yes	612	4	..	1	..	..	..	..	..	...
F	27	No	536	24	2	1	..	..	..	9	..	...
Total or average	51		549	40	9	2	..	..	..	18	..	...

Here it is evident that neither severe nephritis nor calcification of the aorta developed. As all the aortas appeared normal on gross examination, only eighteen were examined histologically. The shortening of the average period of survival of the non-nephrectomized female rats was apparent rather than real, for the reason that these rats were taken from the breeding stock and were killed after they had become unsatisfactory for breeding purposes.

Table 3 gives the findings for the rats fed liver diet 1, the composition of which is as follows:

Liver Diet 1	Percentage
Liver	75
Lard	16
Yeast A	5
Cod liver oil	3
Salt mixture	1

Beef liver is carefully freed of its connective tissue and vessels, cut into small pieces, dried at a moderate temperature, passed through a chopper, dried again at a moderate temperature and then pulverized. This diet is the one used by Newburgh and Curtis.[1] The salt mixture used in this and other diets is that of Osborne and Mendel.[5]

TABLE 3.—*Summary of the Data for the Rats Fed Liver Diet 1*

				Nephritis						Aorta		
Sex	Number of Rats	Nephrectomy	Average Length of Life, Days	0	1+	2+	3+	4+	3+ or 4+, %	Number Examined	Number Calcified	Calcified, %
M	9	Yes	402	..	..	..	..	9	100.0	..	..	....
M	17	No	547	..	2	..	6	9	88.2	16	2	12.5
F	4	Yes	487	..	..	1	1	2	75.0	..	..	....
F	10	No	538	1	4	..	3	2	50.0	10	..	....
Total or average	40		506	1	6	1	10	22	80.0	26	2	7.7

Marked involvement of the kidney had a high incidence in all groups of animals fed this diet, with the nephrectomized animals showing a greater proportion of marked nephritis than the non-nephrectomized animals. It was noticeable that abnormality of the kidneys occurred much more frequently among the rats fed this liver diet than among the controls on stock diet 2 (97.5 per cent as contrasted with 20 per cent).

Calcification of the aorta was noted occasionally in the animals fed liver diet 1. The survival period of the nephrectomized animals was shorter than that of the rats with both kidneys. Severe nephritis was somewhat less frequent in the females than in the males.

In table 4 are given the data for the rats fed liver diet 12. The composition of this diet is as follows:

Liver Diet 12	Percentage
Liver	30
Corn starch	43
Lard	15
Yeast A	5
Cod liver oil	3
Salt mixture	4

5. Osborne, T. B., and Mendel, L. B.: The Nutritive Value of the Wheat Kernel and Its Milling Products, J. Biol. Chem. **37**:557, 1919.

In the nephrectomized rats in this group marked nephritis developed frequently but less so than when liver diet 1 was used. This difference between the effects of the two diets was greater in the case of the intact rats. Again the males showed nephritis more often than the females. The high incidence of aortic calcification in the nephrectomized males is inconsistent with the observations on rats fed liver diet 1, unless factors other than the dietary factors were concerned.

TABLE 4.—*Summary of the Data for the Rats Fed Liver Diet 12*

				Nephritis						Aorta		
Sex	Number of Rats	Nephrectomy	Average Length of Life, Days	0	1+	2+	3+	4+	3+ or 4+, %	Number Examined	Number Calcified	Calcified, %
M	5	No	326	3	1	..	1	..	20.0	5	..	...
M	13	Yes	421	..	1	1	2	9	84.6	9	5	55.5
F	6	No	530	4	1	1	..	..	....	6	..	...
F	10	Yes	505	1	3	1	5	..	50.0	7	..	...
Total or average	34		449	8	6	3	8	9	50.0	27	5	18.5

Attempts were then made to find a diet on which the females also would consistently show severe nephritis. Accordingly desiccated thyroid was added to the diet to stimulate metabolism. The results of this experiment are given in table 5. The composition of the diets used follows:

Liver Diet 14	Percentage
Liver	75.0
Lard	15.0
Yeast A	5.0
Cod liver oil	3.0
Salt mixture	1.0
Calcium carbonate	0.6
Thyroid, desiccated	0.4

Liver diet 15 is the same as liver diet 14, except that calcium carbonate is increased to 0.8 per cent and thyroid is decreased to 0.2 per cent.

Liver diet 20 is the same as liver diet 14, except that calcium carbonate is increased to 0.9 per cent and thyroid is decreased to 0.1 per cent.

Only nephrectomized females were used. All showed marked renal lesions, with the exception of 1 rat fed liver diet 15, which died after one hundred and fifty-three days. It is interesting that the average survival period in the three groups increased progressively as the quantity of thyroid was lessened (being one hundred and forty-eight days with 0.4 per cent thyroid and five hundred and thirty-nine days with 0.1 per cent). The consistent appearance of calcification of the aorta in animals fed these diets is to be noted also.

Since liver diets so frequently produced nephritis, it was necessary to determine whether nonliver diets also cause this condition. Accordingly the following diets were used:

Casein Diet 1	Percentage
Casein	75
Lard	16
Yeast A	5
Cod liver oil	3
Salt mixture	1

This diet is the same as that used by Newburgh and Curtis.[1]

Casein diet 2 differs in that 37.5 parts of dried milk is substituted for an equal amount of casein in casein diet 1.

Diet 2899	Percentage
Wheat	30.0
Casein	20.0
Corn, yellow	17.0
Peas, green split	15.0
Beans, navy	12.5
Cod liver oil	3.0
Calcium carbonate	1.5
Sodium chloride	1.0

This diet is the one used by Polvogt, McCollum and Simmonds,[6] who found that it induces nephritis in rats.

Diet 2899, modified, is made by mixing corn, peas and beans and allowing them to stand overnight in water. They are next cooked in an autoclave at a temperature of 120 C. for two and one-half or three hours and then dried, pulverized and mixed with the other ingredients of diet 2899.

Diet 2899 A	Percentage
Wheat	30.0
Liver	20.0
Corn, yellow	17.0
Peas, green split	15.0
Beans, navy	12.5
Cod liver oil	3.0
Calcium carbonate	1.5
Sodium chloride	1.0

Diet 2899 A, modified, is made by treating corn, peas and beans as for diet 2899, modified. They are then mixed with the other ingredients of diet 2899 A.

The results obtained with these diets are given in table 6. It will be noted that nephritis did develop in animals on a casein diet, provided it was fed for a sufficient length of time. Reduction of the quantity of casein led to less frequent nephritis. Diet 2899, however,

6. Polvogt, L. M.; McCollum, E. V., and Simmonds, N.: Production of Kidney Lesions in Rats by Diets Defective Only in That They Contained Excessive Amounts of Protein, Bull. Johns Hopkins Hosp. **34**:168, 1923.

whether raw or cooked, failed to induce nephritis. When this diet was fed uncooked, skeletal bone deformities and overgrowth of the incisor teeth occurred. The overgrowth was at times so great that the upper incisors curled backward and upward and, occasionally, pierced the brain cavity. However, when the diet was cooked abnormalities of the bones and teeth failed to develop. From a nutritional standpoint this diet, whether raw or cooked, was not satisfactory for rats kept under our conditions.

TABLE 5.—*Summary of the Data for the Nephrectomized Female Rats Fed Liver Diets Containing Thyroid*

Liver Diet	Thyroid, %	Sex	Number of Rats	Average Length of Life, Days	Nephritis						Aorta		
					0	1+	2+	3+	4+	3+ or 4+, %	Number Examined	Number Calcified	Calcified, %
14	0.4	F	3	148	..	..	..	..	3	100.0	3	1	33.3
15	0.2	F	6	276	1	..	..	1	4	83.3	5	1	20.0
20	0.1	F	9	539	..	..	..	3	6	100.0	8	2	25.0
Total or average.....			18	386	1	..	..	4	13	94.4	16	4	25.0

TABLE 6.—*Summary of the Data for the Rats Fed Other Than Liver Diets*

Diet	Sex	Rats	Average Length of Life, Days	Nephritis						Aorta		
				0	1+	2+	3+	4+	3+ or 4+, %	Number Examined	Number Calcified	Calcified, %
Casein 1.....	M	6	676	1	1	2	2	..	33.3	6	..	..
Casein 1.....	F	4	722	..	1	2	1	..	25.0	4	..	..
Casein 2.....	M	6	694	..	1	4	..	1	16.6	6	..	..
Casein 2.....	F	6	631	3	1	2	..	..	....	6	..	..
2899 and 2899 A	M	17	546	17	..	..	..	..	....	17	..	..
2899 and 2899 A	F	15	518	15	..	..	..	..	....	15	..	..
2899 modified and 2899 A modified	M	11	588	11	..	..	..	..	....	11	..	..
2899 modified and 2899 A modified	F	11	625	11	..	..	..	..	....	11	..	..
Total or average.....		76	596	58	4	10	3	1	5.26	76	..	..

Early in our experimental work we thought it desirable to increase the vitamin content in the rations; therefore, in stock diet 2 we substituted 5 per cent of irradiated yeast B for an equivalent quantity of yeast A. After this change abnormal renal conditions began to appear unexpectedly in the control animals. This led to a comparative study of the effects of irradiated and nonirradiated yeast B, using the following diets:

Yeast diet 1 is the same as stock diet 2 except that an equivalent quantity of irradiated yeast B is substituted for yeast A.

Yeast diet 2 differs from stock diet 2 in that an equal quantity of nonirradiated yeast B is substituted for yeast A.

TABLE 7.—*Summary of the Data for the Rats Fed Yeast Diets*

Yeast Diet	Sex	Number of Rats	Nephrectomy	Average Length of Life, Days	Nephritis						Aorta			Hydronephrosis		Calcification of Kidney		Renal Calculi	
					0	1+	2+	3+	4+	3+ or 4+, %	Number Examined	Number Calcified	Calcified, %	Cases	%	Cases	%	Cases	%
1.....	M	3	Yes	270	..	..	1	2	..	66.7	3	3	100.0	..	....	2	66.7	3	100
1.....	M	6	No	359	6	..	..	..	..	0	6	2	33.3	5	83.3	5	83.3	6	100
1.....	F	3	Yes	303	..	..	1	2	..	66.7	3	1	33.3	..	....	1	33.3	3	100
1.....	F	12	No	497	4	2	5	1	..	8.3	12	..	....	7	58.3	1	8.3	12	100
Total or average.....		24		410	10	2	7	5	..	20.8	24	6	25.0	12	50.0	9	37.5	24	100
2.....	M	4	Yes	360	..	..	..	1	3	100.0	4	..	....	..	....	..	....	..	...
2.....	M	4	No	505	2	1	..	1	..	25.0	3	.	....	..	....	..	....	..	...
2.....	F	3	Yes	483	..	1	1	..	1	33.3	2	..	....	..	....	..	....	..	...
2.....	F	7	No	637	4	1	1	1	..	14.3	7	..	....	1	14.3	..	....	..	...
Total or average.....		18		521	6	3	2	3	4	38.3	16	..	....	1	5.5	..	....	..	...

The results obtained with these diets are given in table 7. It will be noted that nephritis occurred with both diets, especially in the nephrectomized rats. In the intact rats fed irradiated yeast, calcification of the aorta, hydronephrosis, calcium deposits in the renal tissue, renal calculi and crystals of dicalcium hydrogen phosphate in the urine were consistently observed; such abnormalities were absent when non-irradiated yeast was used. These results emphasize the importance of seemingly minor factors in experiments dealing with dietary effects.

STUDY OF THE PATHOGENESIS OF NEPHRITIS

Having found that such extensive alterations in the structure of the kidney followed consistently when rats were fed certain diets, we proceeded to study the pathogenesis of these changes. Groups of nephrectomized animals were given diets known to produce nephritis, and the nephrectomized control animals were fed the innoxious stock diet 2. The animals were then killed at intervals in an attempt to demonstrate the site of the initial lesion in the kidney. The results are given in table 8. The data for the fatty infiltration of the liver also are given to show that this condition occurs commonly in rats fed liver diets. We have found that one substance responsible for this effect is the cholesterol contained in liver.[7] It should be noted that a considerable increase in the amount of hepatic fat, detectable by chemical analysis, may occur without its presence being shown by histologic examination. This fatty infiltration is not present consistently in rats in which nephritis is developing. However, its presence may indicate that the factors producing the renal changes are acting on other organs. Liver diets 1 and 3 were used in this study. The composition of the latter follows:

Liver Diet 3	Percentage
Liver	72
Lard	15
Yeast A	5
Cod liver oil	3
Sodium bicarbonate	3
Calcium carbonate	1
Salt mixture	1

This quantity of sodium bicarbonate in the diet was found to produce a neutral or slightly alkaline urine when fed to rats.

7. Blatherwick, N. R.; Medlar, E. M.; Bradshaw, P. J.; Post, A. L., and Sawyer, S. D.: The Dietary Production of Fatty Livers in Rats, J. Biol. Chem. **103**:93, 1933.

The results of this study show that of the 38 rats on stock diet 2, 32 had normal kidneys, 4 had slight alterations in no way suggestive of nephritis and 2 had marked nephritis. The occurrence of nephritis in these 2 animals is at variance with the rest of the data for this diet and is difficult to explain but may have been due to the presence of irradiated yeast in the diet at the time the two animals received it.

The kidneys of the rats fed the liver diets gave a different picture from that of the ones on the stock diet. Among the rats fed liver,

TABLE 8.—*A Study of the Pathogenesis of Nephritis*

	No. of Rats	Days on Diet	Fatty Infiltration of Liver: 0	Slight	Mild to Moderate	Nephritis: 0	1+	2+	3+	4+
Stock diet 2.....	4	63	4	..	..	4	..	..	..	..
	3	106	3	..	..	3	..	..	..	..
	3	127	3	..	..	2	1	..	..	..
	3	134	3	..	..	3	..	..	..	..
	3	162	3	..	..	2	1	..	..	..
	3	173	3	..	..	3	..	..	..	..
	3	190	2	1	..	2	1	..	..	..
	3	215	3	..	..	3	..	..	..	..
	3	225	..	3	..	2	1	..	..	..
	3	256	3	..	..	1	..	..	..	2
	2	291	2	..	..	2	..	..	..	..
	4	333	4	..	..	4	..	..	..	..
	1	372	1	..	..	1	..	..	..	..
Total or average	38		34	4	..	32	4	..	..	2
Liver diet*.....	4	59	4	..	..	4	..	..	..	..
	3	59	3	..	..	2	1	..	..	..
	3	89	..	1	2	1	2	..	..	..
	3	105	..	3	..	..	3	..	..	..
	3	121	..	..	3	..	3	..	..	..
	3	145	..	3	..	..	3	..	..	..
	3	151	..	2	1	1	..	3	..	..
	3	180	..	1	2	..	2	1	..	..
	3	190	3	..	..	..	..	1	1	1
	2	212	..	..	2	..	1	1	..	..
	3	228	3	..	..	..	1	1	..	1
	2	270	2	..	..	..	1	1	..	..
Total or average	35		15	10	10	8	17	7	1	2

* In this part of the table liver diets 1 and 3 are not designated, since other experiments showed that both were effective in producing nephritis. Although the presence of sodium bicarbonate and calcium carbonate in liver diet 3 rendered the urine neutral or slightly alkaline, the development of nephritis was not retarded.

only 8 had normal kidneys; 17 showed abnormalities not suggestive of nephritis; 7 showed mild, 1 moderate, and 2 severe nephritis. Prior to one hundred and fifty days there was no evidence to suggest that general nephritis would eventually develop; after that time the results were not sufficiently uniform to fix the site of the initial lesion in the kidney. From the evidence we conclude that the functional derangement of the kidney antedates considerably the appearance of the histologic changes characteristic of nephritis, but once the latter changes begin frank and severe nephritis develops quickly.

THE HISTOLOGIC PICTURE OF THE NEPHRITIC KIDNEY

The essential changes in the kidney occur in the glomeruli and tubules. The glomerular changes begin as focal hyalinization in the capillary tuft and end in complete obliteration of the capsular space. Inflammation is never marked, but when present it is shown by the infiltration of monocytes and an occasional lymphocyte. The capillary tuft commonly contains demonstrable fat, which is scattered throughout the structure either in small isolated foci or in considerable quantities. The origin of this fat is obscure, but there is a suggestion that it may come from the circulating blood and may be embolic. The number of glomerular units involved varies with the extent of the nephritis—from a few in cases of mild nephritis to many in cases of severe nephritis. In the latter cases the glomeruli show varying degrees of fibrosis, with some units apparently uninvolved and retaining their normal structure. Evidently the number of functional glomeruli becomes greatly decreased as the disease progresses.

The changes in the tubular epithelium appear to be limited, in the early stages at least, to the distal portion of the tubule. In this region the earliest changes observed are the presence of demonstrable fat and, with certain diets, especially liver 1, a considerable quantity of pigment the nature of which is not understood. This pigment does not contain iron and in some instances stains faintly with sudan III stain. It is not a constant feature, as some of the kidneys in which severe nephritis was present contained practically none. Constant features are the enlargement of the epithelial cells of the distal tubule and their staining a more intense blue than in the normal kidney. The latter change is not observed in the proximal tubules, for there the cytoplasm retains its pink-staining characteristics. Evidently, hyperplasia of the epithelium of the distal tubules occurs frequently. Mitotic figures may be present at certain stages, and often the entire lumen of the distal portion of the tubule is enlarged and filled with well preserved epithelial cells. At a later stage there is disintegration of the epithelial cells, always accompanied with infiltration of lymphocytes, which may be marked. Fibrosis occurs concurrently with or following these changes, and the final histologic picture is that of scar tissue formation without tubular epithelium and with little lymphocytic infiltration.

As hyperplasia, disintegration of the distal tubular epithelium and fibrosis occur, there appears to develop a damming back of the urinary secretion into the proximal convoluted tubules. This leads to dilatation of the tubules, with the formation first of granular débris and later of hyaline casts. The end-result is cystic dilatation of the tubules, within which are large numbers of casts, some containing demonstrable fat. There is little evidence of hyperplasia of the epithelium in the proximal

convoluted tubules, which in instances of marked distention may be completely disintegrated. The epithelial cells may or may not contain fat or pigment.

There appear to be no consistent and significant changes in the walls of the arteries in these kidneys; that is, the development of nephritis seems to be unrelated to vascular changes, exclusive of those which may occur in the glomerular tuft.

It may be interesting to consider the significance of calcification of the aorta as encountered in our experimental rats. The histologic picture shows calcium, either in plaques or in diffuse deposits, in the median portion of the artery. Otherwise, the artery appears normal. No evidence of an inflammatory reaction and no semblance of the process of arteriosclerosis, as seen in man, were noted. We have encountered 21 instances of calcification of the aorta; 6 cases were in a group of 24 rats on a diet which contained 5 per cent irradiated yeast, 4 cases occurred in a group of 18 rats fed a liver diet containing thyroid and the 11 remaining cases were found at random in groups of animals on other liver diets. Dietary factors possibly bear directly on calcification of the aorta, as this condition has not been observed in the examination of several hundred of our rats on nonnephritis-producing diets. Just what these factors are and how they induce calcification is not understood. An examination of the data on the blood indicates clearly that hypercholesteremia was no more frequent in the rats with calcification of the aorta than in those without it.

Our interpretation of the pathologic processes described is as follows: The glomerular changes are probably dependent on an alteration in the constituents of the blood. This produces a change in the filter apparatus, eventuating in complete obliteration of the functional glomerulus by fibrosis. The altered filtration allows passage into the urine of substances which profoundly affect the epithelium of the distal renal tubule. Hyperplasia and later disintegration of these cells then occur and are followed by lymphocytic infiltration and fibrosis. A damming back of urinary secretion results. The final picture (fig. 1) resembles that seen in man in so-called chronic atherosclerotic nephritis, but without the presence of atherosclerosis. We suggest that the whole picture represents a phenomenon due to overwork of the distal portion of the renal tubule resulting from changes in the filtering ability of the glomerular tuft.

URINARY OBSERVATIONS

In man the laboratory recognition of nephritis rests on the presence, quantity and persistence of albumin in the urine, together with the occurrence of casts and red blood cells in the sediment; on evidence in the blood of the retention of nitrogenous substances, and, in certain

conditions, of a lowered albumin-globulin ratio and an increased cholesterol content, and on functional renal tests, such as fixation of the specific gravity, the urea clearance and the ability to excrete phenolsulfonphthalein. The presence or absence of such phenomena in the

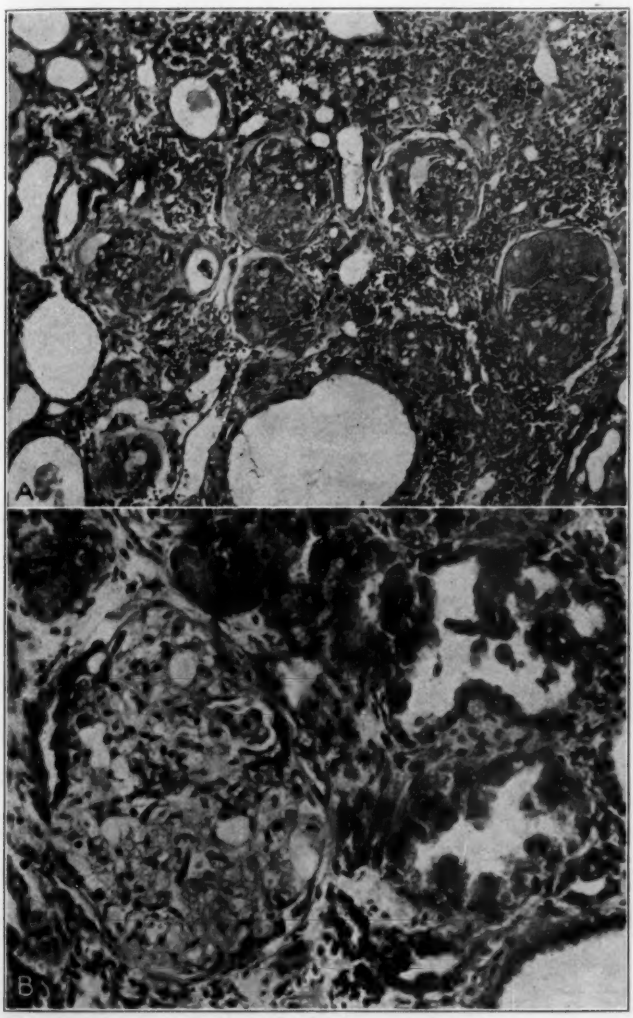

Fig. 1.—*A*, photomicrograph, × 300, showing the type of nephritis encountered. This is regarded as 4 plus nephritis in our classification. *B*, a photomicrograph of higher power, × 600. Note the sclerosing glomerulus and the hyperplastic epithelium of the distal renal tubule.

rat must be determined if one is to learn whether the pathologic process in this animal resembles the manifestations in man. We have accumulated data for the urine relative to the albumin, casts and other formed elements and for the blood relative to the retention of nitrogenous substances, the albumin-globulin ratio and the cholesterol content.

In tabulating the data for the urine and blood the rats have been considered in the following groups: (*a*) rats without nephrectomy which showed normal kidneys on histologic examination, (*b*) rats with one kidney removed and with the remaining kidney histologically normal, (*c*) rats without nephrectomy which had 3 plus or 4 plus nephritis histologically and (*d*) rats with one kidney removed and with 3 plus

TABLE 9.—*Albumin and Casts in the Urine of Intact and Nephrectomized Rats with Normal Kidneys or with 3+ or 4+ Nephritis*

				Albumin in Urine				Casts in Urine		
No. of Rats	Sex	Nephrectomy	Condition of Kidney at Necropsy	No. of Examinations	Over 0.3 %	Over 0.1 %	Positive, %	No. of Examinations	No. of Positive Examinations	Positive Examinations, %
20	M	No	Normal	287	24	...	8.4	291	25	8.6
20	F	No	Normal	280	...	19	6.8	287	13	4.5
13	M	Yes	Normal	124	67	...	54.4	126	32	25.0
17	F	Yes	Normal	235	...	86	36.6	236	92	39.0
20	M	No	3+ or 4+ nephritis	342	268	...	78.3	327	196	60.0
7	F	No	3+ or 4+ nephritis	115	...	38	33.3	120	45	37.5
20	M	Yes	3+ or 4+ nephritis	242	199	...	82.0	244	181	73.8
20	F	Yes	3+ or 4+ nephritis	309	...	226	70.0	316	229	72.4
5*	M	No	Hydronephrosis	47	0	...	0.0	48	18	37.5
6*	F	No	Hydronephrosis	86	...	15	17.4	87	47	54.0
4†	M	No	Normal	45	0	...	0.0	45	5	11.1
5†	F	No	Normal	89	...	8	9.0	90	24	26.6

* Irradiated yeast diet.
† Nonirradiated yeast diet.

or 4 plus nephritis in the remaining organ. We found it advisable to subdivide the groups according to sex as far as the urine was concerned, since the albumin content of the urine is normally greater in the male than in the female, as has been shown by Bell [8] and Shih.[9] The latter stated his conclusion that most of this excess protein originates in the genital tract. That such may be the case is indicated by the usual presence of large numbers of spermatozoa in the urine of our male rats.

In table 9 are assembled the data for albumin and casts obtained from over 2,000 urinalyses. After careful study of the results of the

8. Bell, M. E.: Albuminuria in the Normal Male Rat, J. Physiol. **79**:191, 1933.

9. Shih, H. E.: The Origin of Protein in the Urine of Albino Rats, Am. J. Physiol. **113**:120, 1935.

examinations of normal rats, we have been led to consider as abnormal an albumin content over 0.3 per cent in the male and over 0.1 per cent in the female rat and the presence of any casts. An increase in the albumin content or the presence of casts in intact rats with histologically normal kidneys did occur but was rare. In nephrectomized rats, with the remaining kidney histologically normal, there occurred a significant increase in the abnormal albumin values and the presence of casts. Rats having moderate or severe nephritis showed a much higher incidence of abnormal albumin values and of casts than did the nonnephritic animals. This is true for both nephrectomized and nonnephrectomized males and females. Rats which showed hydronephrosis on diets containing irradiated yeast showed a marked incidence of casts, with a slight increase in the albumin content; animals on diets containing nonirradiated yeast showed a similar though less marked picture. Male rats showed a higher percentage of abnormal findings than females in all groups except those on the yeast diets, in which the reverse was true.

The data relative to albumin, casts and the albumin-globulin ratio obtained for the rats used in the study of the pathogenesis of nephritis are given in table 10. Here a marked increase in the abnormal values will be noted in all groups. The rats on the liver diets showed many more abnormal values than did those on stock diet 2.

To demonstrate the evolution of the changes in the albumin content of the urine, we have constructed figures 2 to 5. In these graphs the curves represent the averages for the monthly albumin values of all the animals within a group. The number of animals used and the group curves are given in the graphs.

Figures 2 and 3 show clearly the marked differences in behavior between male and female rats. It will be noted that all groups started with normal values. Increased albumin values occurred earlier and reached higher maxima in groups of male rats than in the corresponding groups of females.

Figure 4 is drawn from the data given in table 5 and shows the striking effect of thyroid when added to a nephritis-inducing diet. The rapid onset and evolution of the disease when 0.4 per cent thyroid was given were in marked contrast to the gradual change that occurred with 0.1 per cent thyroid. The average length of life of the animals was one hundred and forty-eight days with 0.4 per cent thyroid and five hundred and thirty-nine days with 0.1 per cent thyroid. All the data indicated that the feeding of the larger quantity of thyroid resulted in producing severe nephritis, which we believe contributed significantly to shortening the average length of life.

TABLE 10.—*Data on the Pathogenesis of Nephritis: Albumin and Casts in Urine and Albumin-Globulin Ratio of the Blood*

Diet	Sex	No. of Rats	Nephrectomy	Albumin in Urine				Casts in Urine			Albumin-Globulin Ratio of Plasma		
				No. of Examinations	Over 0.3 %	Over 0.1 %	Positive, %	No. of Examinations	No. of Positive Examinations	Positive Examinations %	No. of Examinations	Ratio Less Than 1	Ratio of Less Than 1 %
Stock 2	M	17	Yes	98	61	..	62.2	98	35	35.7	16	6	37.5
Stock 2	F	22	Yes	127	..	23	12.3	127	32	25.2	21	1	4.8
Liver	M	15	Yes	82	71	..	86.6	86	51	59.3	15	10	66.7
Liver	F	20	Yes	110	..	37	33.6	113	43	38.1	17	6	35.3

In figure 5 it will be noted that in nephrectomized rats the liver diets produced a more rapidly developing and a more severe albuminuria than did the stock diet.

The first casts to appear in the urine of rats were often pigmented. Granular casts were commonly present in cases of developing nephritis. Later, hyaline casts were present, and in the final stages highly refractive casts appeared. Cylindroids were often associated with the casts. Red blood corpuscles were not present except in the case of rats fed yeast diet 1. It will be recalled that all these animals had renal calculi. Crystals of dicalcium hydrogen phosphate were commonly present in the urinary sediment of rats fed irradiated yeast, but they were never observed in the specimens from rats given other diets.

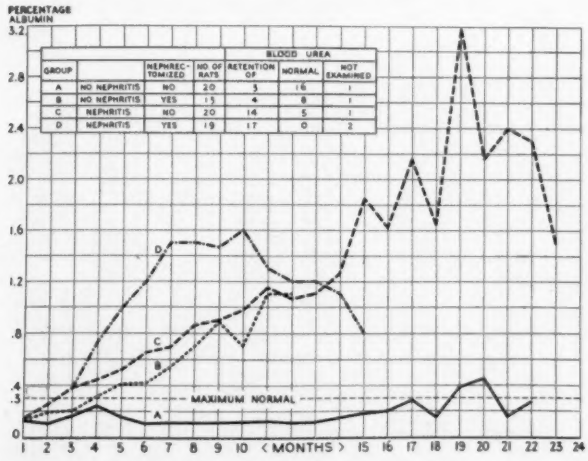

Fig. 2.—Chart showing the excretion of albumin and the retention of urea in male rats with 3 plus or 4 plus nephritis and in male rats without nephritis.

BLOOD PICTURE

Urea Retention.—We have considered 30 mg. per hundred cubic centimeters as the upper limit of normal for the urea nitrogen content of the blood of the rat. Thirty-nine rats which were found to have 3 plus nephritis gave an average value of 61.4 mg., the highest value being 221 mg. per hundred cubic centimeters. Twenty-three (59 per cent) of the values were above the normal limit. In the case of 85 rats with 4 plus nephritis, the average value was 141 mg., and the highest value was 375 mg. per hundred cubic centimeters. Not a single case of nonretention was observed in this series. It is evident that the histologic classification of the extent of nephritis was in accord

with the degree of urea retention. Among the 40 intact rats without nephritis, there were 3 (7.5 per cent) instances of retention. Six (20 per cent) of 30 nephrectomized animals without nephritis showed retention. This difference is significant and indicates that one kidney has difficulty in performing adequately the function of forming normal urine. The observations, as a whole, show that renal injury must be severe before retention of nitrogenous products consistently occurs.

Albumin-Globulin Ratio.—In certain types of nephritis the quantities of albumin and globulin in the blood plasma are changed so that this ratio is decreased. Such a change is found in the chronic active stage of hemorrhagic nephritis and is marked in cases of nephrosis. We have considered values for this ratio of less than 1 to be abnormal.

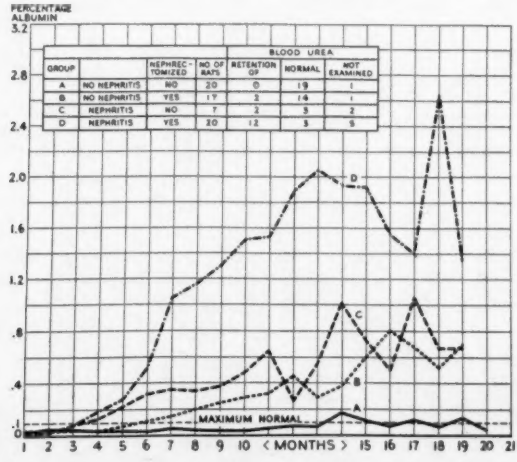

Fig. 3.—Chart showing the excretion of albumin and the retention of urea in female rats with 3 plus or 4 plus nephritis and in female rats without nephritis.

In a series of 22 rats with 3 plus or 4 plus nephritis, all but 1 showed an abnormal ratio. Our study of the pathogenesis of nephritis revealed that only 1 (4.8 per cent) of 21 female rats fed stock diet 2 had a ratio of less than 1, while 6 (35.3 per cent) of 17 had a low ratio when fed the liver diets. Six (37.5 per cent) of 16 male rats fed stock diet 2 had an abnormal ratio, and 10 (66.7 per cent) of 15 had an abnormal ratio when fed liver. Apparently, the albumin-globulin ratio is more easily disturbed in males. Several cases of inversion of the ratio in rats without nephritis were observed, and this indicates that factors other than nephritis influence the concentration of protein in the blood.

Cholesterol.—The cholesterol content of the blood plasma has been observed to increase in conditions in which there is an inversion of

the albumin-globulin ratio. It is believed that this increase in the cholesterol content is the result of an effort to maintain the normal osmotic relationships of the blood. We have found it necessary to establish two maximum normal limits for the cholesterol content of the blood of our rats, owing to the fact that the cholesterol contained in liver diets increases the content of this substance in the blood. The average value for the plasma cholesterol of 31 rats without nephritis which were fed stock diet 2 was 88 mg. per hundred cubic centimeters, the mean deviation being 29.2 mg. Therefore, it has been considered that values over 146 mg. per hundred cubic centimeters for rats fed liver-free diets indicate hypercholesteremia. Similarly, 88 rats without nephritis which were fed liver diets gave a mean value of 125.5 mg.

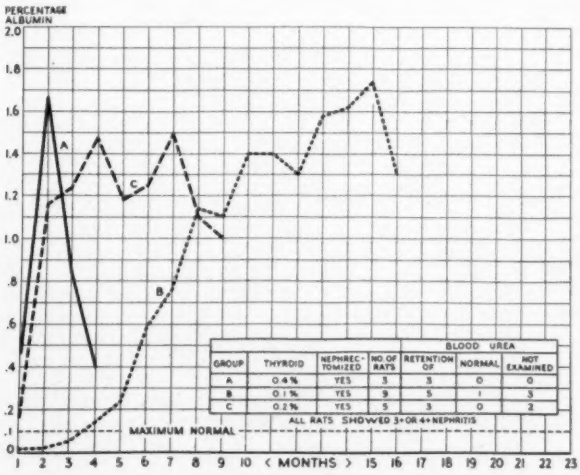
PERCENTAGE ALBUMIN
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
.1
0
A
B
C
MAXIMUM NORMAL
1 2 3 4 5 6 7 8 9 10 < MONTHS > 15 16 17 18 19 20 21 22 23
BLOOD UREA
GROUP | THYROID | NEPHREC-TOMIZED | NO. OF RATS | RETENTION OF | NORMAL | NOT EXAMINED
A | 0.4% | YES | 3 | 3 | 0 | 0
B | 0.1% | YES | 9 | 5 | 1 | 3
C | 0.2% | YES | 5 | 3 | 0 | 2
ALL RATS SHOWED 3+ OR 4+ NEPHRITIS

Fig. 4.—Chart showing the excretion of albumin and the retention of urea in female rats fed liver diet plus thyroid.

per hundred cubic centimeters. Twice the mean deviation of 46.7 mg. indicates that 219 mg. per hundred cubic centimeters should be taken as the upper limit for the normal cholesterol content when liver diets are concerned. The results for rats fed nonliver diets were as follows: 5 (50 per cent) of 10 with 3 plus nephritis showed hypercholesteremia, and 7 (87.5 per cent) of 8 with 4 plus nephritis showed values above the normal limit. The following results were obtained for the animals fed diets containing liver: Fifteen (41.7 per cent) of 36 having 3 plus nephritis had hypercholesteremia, and 34 (51.5 per cent) of 66 with 4 plus nephritis showed values above normal. The values for rats with nephritis were in general agreement with the changes observed in the albumin-globulin ratio.

In our study of the pathogenesis of nephritis 4 (11.1 per cent) of 36 rats fed stock diet 2 had hypercholesteremia; 2 of these had 4 plus nephritis, with retention of nonprotein nitrogen, and the others had no nephritis and showed no retention. Three (9.4 per cent) of 32 animals fed the liver diets accumulated cholesterol in the blood; 2 of these had retention of nonprotein nitrogen and 2 plus nephritis, and the other with 1 plus nephritis had no retention.

If the values for cholesterol are considered in relation to calcification of the aorta, it will be seen that 18 animals with calcification of the aorta gave an average value of 182 mg. of cholesterol per hundred cubic centimeters of blood, the range being from 87 to 290 mg. These results show that the calcification was not due to an abnormally high cholesterol content of the blood.

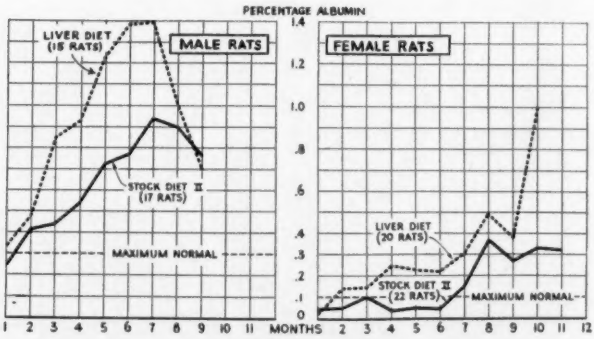

Fig. 5.—Chart showing the excretion of albumin in nephrectomized male and female rats fed liver diets 1 and 3 and stock diet 2.

COMMENT

The present concept of the etiology of chronic nephritis is necessarily in a confused state. One group of workers think that infection superimposed on a preexisting state of allergy initiates the degenerative process; another group, in which are Newburgh and his associates, believe that dietary factors play an essential rôle. That the nephritis produced experimentally in our rats was not dependent on the existence of infectious processes is evident from the following observations: (*a*) The organs most frequently infected were the lungs, but there was no higher incidence of nephritis in animals with marked pulmonary involvement than in those free from it; (*b*) in the rats on diet 2899 local infection in the mouth secondary to erosion of the mucosa by overgrowth of the upper incisors occurred often, but there was no nephritis among these animals; (*c*) in the group of rats in

which hydronephrosis developed while they were receiving a diet containing irradiated yeast practically no renal infection and but little nephritis were present, although urinary retention was sometimes marked and was often accompanied with inflammation of the wall of the bladder and with severe ulceration of the mucosa induced by calculi.

Our results herein reported have shown conclusively that diet alone will produce nephritis and will also determine its severity. We cannot at present explain how the diet produces these effects.

That the injurious principle is not confined to the protein content of the diet may well be. From a purely theoretical point of view certain diets may possibly aid in establishing abnormal complex metabolic processes which lead to the formation of abnormal products or of increased normal end-products, to the overfiltration of the normal end-products, to the excretion of the abnormal products or to the retention of certain products. Whatever these substances are that pass the glomerular filter, it is conceivable that they may have existed as such in the diet or that they may have been produced within the body during metabolism. From the urinary findings it seems that an abnormal functional condition of the kidney considerably antedates the appearance of histologic evidence of nephritis.

From our experience it is evident that the greatest care must be used in the planning and compounding of experimental diets, for the overlooking of a seemingly minor factor may lead to unsuspected complications and to erroneous conclusions relative to the effects produced. Thus the cooking of diet 2899 eliminated the development of abnormalities of the teeth and bones; the substitution of irradiated for non-irradiated yeast led to hydronephrosis, calcification of the aorta and the formation of renal calculi, and the addition of thyroid to the diet favored the production of nephritis and of calcification of the aorta. By reducing the quantity of thyroid the development of nephritis was delayed, and the average length of life of the rats was increased. Further studies on the effects of the administration of thyroid are now in progress. Obviously, a diet that has been proved to be satisfactory for the growth and well-being of the type of animal used should always be employed as a control.

In such experimental work as ours attention must be paid to the gross and microscopic examination of the various types of tissue and must not be limited to a consideration of a single organ, such as the kidney or liver. This became apparent early in these studies, when we found that during the first few weeks we could identify the rats fed liver diet 1 by the fatty infiltration in the liver while the kidneys appeared normal. Abnormalities in other organs cannot always be

correlated with the most important pathologic process produced, but they tend to show that the body as a whole may be involved rather than any one tissue or organ.

A peculiar and interesting fact which we discovered is that the female rat is more refractory to the production of nephritis by diet than the male. This was well shown by intact rats fed identical diets; in 21 per cent of the males marked nephritis developed, while only 6 per cent of the females showed a similar condition. Whether this difference is confined to the rat is unknown. May it not pertain to the sexes in other species? Sex differences in the glycogen content of the tissues and in the metabolism of fat also have been observed. Nature may have evolved distinct metabolic differences between the male and the female, perhaps to enable the latter better to fulfil her special functions.

The extreme disorders of calcium metabolism induced by diets containing irradiated yeast deserve special consideration. Other investigators have reported the formation of renal calculi and the deposition of calcium in the tissues resulting from the administration of toxic doses of vitamin D. However, we were rather surprised to find that such changes could be produced so consistently by the quantity of irradiated yeast fed to our rats. These observations suggest that the indiscriminate use of the many irradiated products now available may be the source of unfortunate complications later.

The changes noted in the blood constituents were in general agreement with those observed in human beings with nephritis. It was found that retention of urea did not occur until injury to the kidney had become severe. Our data for the albumin-globulin ratio showed it to be disturbed in a large majority of the rats with severe nephritis, and there were indications that it is more easily changed in male than in female rats. The observed increases in the cholesterol content of the blood harmonized with the concomitant decreases in the albumin-globulin ratio. Rats in which calcification of the aorta had developed did not have hypercholesteremia. Unfortunately, none of these determinations is useful in detecting early functional changes in the kidney.

Our data suggest that irreparable renal damage occurs only as the end-result of an interaction of unknown factors which have existed for a considerable period of time. These unknown factors, our ignorance of how they are interrelated and the uncertainty concerning the changes which occur between the initiation of the process and the appearance of the irreparable damage greatly hinder at this time an explanation of the pathologic process. If one considers the evidence of functional derangement of the kidney, as shown by the appearance of abnormal quantities of albumin and of casts in the urine, it is apparent that the

functional impairment antedates the appearance of histologic changes by a considerable period. If it is assumed that the initial impairment occurs in the functional activity of the glomerulus as a filter, it may be deduced that the tubular epithelial cells, in their efforts to conserve portions of the filtered plasma, eventually reach a state in which they in turn become functionally overworked; when such a condition is reached they apparently disintegrate. At about this time an inflammatory reaction ensues, as evidenced by lymphocytic infiltration and fibrosis. The accumulation in the tubule of material which has passed the glomerular filter occurs later, after the periods of epithelial hyperplasia, inflammation and fibrosis. It is somewhat uncertain just where in the tubule the epithelium suffers the most damage, but from the location of the cellular hyperplasia, the staining reaction of the epithelial cells and the retention of urinary secretion the injury seems to be restricted principally to the distal portion. Why the distal portion should be impaired rather than the proximal portion is not clear; the answer to this question must be deferred pending a better understanding of the function of the epithelium along the course of the tubule. Our observation that in rats the irreparable damage is restricted to a certain portion of the tubule is in agreement with Richards'[10] observation that in the frog and in Necturus the epithelium of the proximal portion differs functionally from that of the distal end.

The data presented clearly demonstrate that casts and abnormal quantities of albumin appeared earlier in nephrectomized rats in which severe nephritis ultimately developed than in the animals in which nephritis failed to develop; the output of albumin was greater, and there was a tendency for it to decline toward the end of the disease, probably because of the destruction of many glomeruli. These same phenomena were present in the intact rats, but in these they occurred later and less abruptly. It should be noted that casts were at times absent from the urine even when severe nephritis was present; certainly the number of casts was no true index of the severity of the nephritis, as it varied from time to time while the disease was actively progressing.

The significance of the abnormal urinary findings deserves special consideration. It is apparent that an increase in the albumin content or the presence of casts does not necessarily indicate pathologic nephritis. Such findings do indicate a functional impairment of the kidney which may or may not terminate in irreparable renal damage. In nephrectomized animals fed stock diet 2, with persistent evidence of functional impairment, severe nephritis did not develop, even though the animals lived for two years after the operation. The influence of

10. Richards, A. N.: Urine Formation in the Amphibian Kidney, Harvey Lectures, 1934-1935, Baltimore, Williams & Wilkins Company, 1936, p. 93.

diet on the functional activity of the kidney is clearly shown in figure 5. Here the function of the remaining kidney was disturbed in all groups, but the nephritis-inducing diets caused a greater functional disturbance than did the harmless stock diet 2. Obviously, a single kidney cannot carry the functional load as well as the two organs which nature has provided. It is to be hoped that a method may be found by which it will be possible to recognize functional impairment before permanent structural damage has occurred, since it may be possible to cure the former, even though it may not be possible to prevent further degeneration in a kidney once permanent damage has been established.

SUMMARY

Chronic nephritis has been produced in rats by feeding them diets high in protein. The evidence presented has shown that functional impairment of the kidney may exist for some time before histologic changes indicative of nephritis become apparent. The excretion of albumin or the presence of casts in the urine was interpreted as indicating functional impairment of the kidney, although not necessarily proving the presence of nephritis. When irreparable damage occurred, retention of nonprotein nitrogen, inversion of the albumin-globulin ratio and hypercholesteremia were observed.

It was found that female rats were more refractory to the production of nephritis by diet than males. The addition of desiccated thyroid to the diet of nephrectomized females favored the development of nephritis. The feeding of irradiated yeast led to hydronephrosis, calcification of the aorta and the formation of renal calculi. Calcification of the aorta was not associated with hypercholesteremia.

We have suggested that the initial impairment of the kidney may be a change in the functional activity of the glomerulus. The filtering of unusual substances into the tubule throws an additional strain on the distal epithelial cells, causing them finally to disintegrate. An inflammatory reaction ensues. Material which passes through the glomerulus is then dammed back into the proximal tubule and causes cystic dilatation. Judging from the location of the cellular hyperplasia, the staining reaction of the epithelial cells and the retention of urinary secretion, the primary injury to the tubular epithelium seems to be limited principally to the distal portion. The entire process may be looked on either as a result of overwork or as a toxic phenomenon.

The injurious factor may exist as such in the diet, or it may be produced during metabolism. If the harmful substance exists in the diet, it may not necessarily be confined to the protein fraction. The nephritis may be induced by an abnormal excretion of normal end-products, by an excretion of abnormal products or by the retention of certain products.

ACUTE VEGETATIVE ENDOCARDITIS CAUSED BY BACILLUS DIPHTHERIAE

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Acute vegetative endocarditis caused by the diphtheria bacillus is of infrequent occurrence, the literature up to the present time containing reports of only six cases. The micro-organisms isolated in three of these cases had all the cultural and morphologic characteristics of Bacillus diphtheriae but did not produce a toxin in amounts sufficient to be lethal for guinea-pigs. In the other three cases reported the bacilli were characteristic of B. diphtheriae morphologically and culturally as well as in their lethal effect when broth cultures were injected into guinea-pigs.

The nontoxic bacilli simulating B. diphtheriae were isolated at autopsy in the cases reported by Howard [1] in 1893 and by Roosen-Runge [2] in 1903 and 1925. Herzog [3] in 1918 was the first to isolate a strain of B. diphtheriae from the blood in pure culture which was lethal for guinea-pigs when 1 cc. of a three day broth culture was inoculated subcutaneously. The bacilli isolated from the two patients reported on by Chiari [4] in 1933 and 1935 also were typical in their lethal effect on guinea-pigs when broth cultures were inoculated subcutaneously. A patient with this type of endocarditis came to autopsy recently at the Vanderbilt Hospital, and a detailed report of the case follows.

REPORT OF A CASE

The patient, a 16 year old boy, was admitted to a medical ward four months before his death. He complained of frequent, moderately severe nosebleeds,

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1. Howard, W. T., Jr.: Acute Ulcerative Endocarditis Due to the Bacillus Diphtheriae, Bull. Johns Hopkins Hosp. **4**:32, 1893.

2. Roosen-Runge: Ein Fall von Diphtheriebazillensepsis, München. med. Wchnschr. **50**:1252, 1903; Ueber Bakteriämie durch Diptheriebazillen, Virchows Arch. f. path. Anat. **254**:379, 1925.

3. Herzog, G.: Demonstration einer ulzerozen Endokarditis durch Diphtherienbazillen, München. med. Wchnschr. **65**:29, 1918.

4. Chiari, H.: Zur Frage der Diphtherie-Endokarditis, Centralbl. f. allg. Path. u. path. Anat. **58**:193, 1933; Zur Frage des Uebertretens der Diphtheriebazillen in die Blutbahn und ihrer Ansiedlung in inneren Organen, Wien. klin. Wchnschr. **48**:685, 1935.

which had their onset about three months previous to his admission to the hospital. He had an attack of rheumatic fever at the age of 7 years without any appreciable sequelae. His father and one uncle had had spontaneous nosebleeds over a period of one year during adolescence.

Physical examination revealed that the boy's heart was moderately enlarged to the left, the spleen was palpable and there were numerous cutaneous telangiectases scattered over the body. Studies of the blood revealed nothing abnormal, except a low platelet count, from 40,000 to 130,000 per cubic millimeter. The bleeding time, clot retraction and tourniquet tests were normal. No definite diagnosis as to the cause of the nosebleeds could be made during the patient's first stay of two months in the hospital. During this time he had a few mild nosebleeds, which were controlled by packing. He had a low grade continuous fever. He was sent home and remained there for six weeks. He continued to have occasional mild nosebleeds. Two days before his second admission to the hospital a severe nosebleed occurred which necessitated packing. Attempts to remove the pack brought on severe bleeding, so that it had to be replaced. At that time the nasopharynx was tender, and the pack had a foul odor. Other physical and laboratory findings were much the same as at the previous entry, except that gradual enlargement of the spleen and liver was observed. During the next ten days he had three severe nosebleeds, which were controlled by packing. Five days before his death the temperature suddenly rose from 102 to 105 F. Culture of the blood was positive for an unidentified gram-positive bacillus, which grew 142 colonies per cubic centimeter. Enlargement of the heart to the left rapidly developed together with ascites. A systolic and diastolic murmur developed. During the last twenty-four hours numerous petechial hemorrhages appeared over the body. The patient was anuric for thirty-six hours preceding death. The nonprotein nitrogen content of the blood rose to 92 mg. per hundred cubic centimeters.

At autopsy the heart weighed 440 Gm. There were moderate hypertrophy and dilatation of the left ventricle. A large friable fibrinous vegetation extended along the edges of the mitral valve (fig. 1 *A*). The leaflets of the valve were scarred, and their edges were rolled and thickened. Direct smears from the vegetation showed innumerable long, slender bacilli, which were irregular in shape and form and showed a tendency to be arranged in parallel rows. They were chiefly gram-positive, but many of them decolorized readily (fig. 1 *C*). Stained with Löffler's methylene blue, metachromatic bipolar granules, characteristic of the diphtheroids, could be demonstrated. Microscopic section of the valve showed the valvular endothelium to be ulcerated, and from its edge extended a dense thrombotic mass, consisting chiefly of micro-organisms bound together by fibrin and a few polymorphonuclears (fig. 1 *B*). In sections stained by the Gram method these organisms were not decolorized.

Other significant features were noted microscopically. There was acute arteritis in the smaller vessels of the heart, lungs, intestine and skin, characterized by hyaline necrosis of the intimal layer and perivascular collections of polymorphonuclears and monocytes. Occasionally a hyalinized thrombus was seen within the lumen of an arteriole. There was acute splenitis characterized by hyaline necrosis of the centers of all the malpighian corpuscles. Acute embolic nephritis and acute interstitial orchitis also were present. There were marked hemorrhages in the adrenal capsules.

Growth Characteristics of the Micro-Organism.—A pure culture of the bacillus was obtained from the heart blood, the mitral vegetation and the spleen under aerobic and anaerobic conditions. The organisms appeared in all instances as

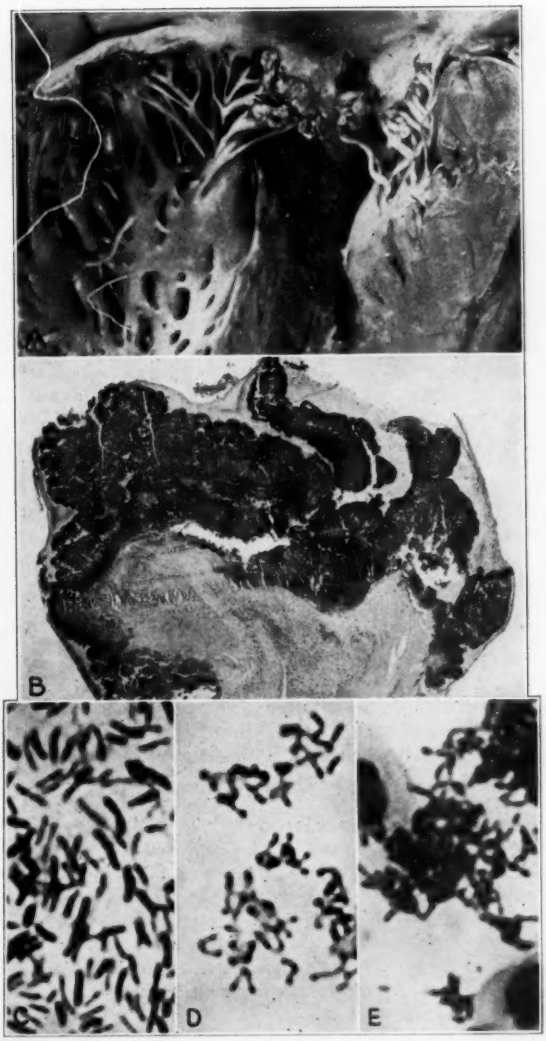

Fig. 1.—*A*, section of the heart, showing ulceration and vegetation of the mitral valve. *B*, section from the mitral valve showing extensive vegetation, consisting chiefly of micro-organisms. *C*, smear preparation from the mitral vegetation showing micro-organisms. Gram stain; × 2,000. *D*, smear preparation from a twenty-four hour broth culture stained by the Neisser method, showing metachromatic bipolar granules; × 2,000. *E*, smear preparation from a twenty-four hour culture of the organism on the chorio-allantoic membrane of a chick embryo. Wright stain; × 2,000.

pleomorphic, short, fat bacilli, which tended to be arranged in picket fence formation. Stained by the Gram method, the organisms were easily decolorized. Many gram-negative forms were present, although the majority were gram-positive. A twenty-four hour broth culture showed granular growth which settled to the bottom of the tube, but by subsequent culture the organisms were easily induced to form a pellicle on the surface of this medium. Subcultured on Löffler's medium, a twenty-four hour culture showed numerous grayish white, glistening colonies. Colonies on 10 per cent blood agar showed no hemolysis. After twenty-four hours small, round, opaque, white, glistening colonies appeared, each with the center heaped-up and the entire edge smooth. The bacillus fermented dextrose, lactose and maltose, with acid and without gas. No fermentation occurred in sucrose. Smears from these subcultures showed pleomorphic granular bacilli which had an irregular staining capacity. By the Gram method they were readily decolorized, but when stained by the Neisser method metachromatic bipolar granules were readily demonstrated (fig. 1 *D*). A tendency to arrangement in parallel rows, curved forms and clubbed ends was evident.

The organisms cultured on artificial mediums, however, showed marked differences from those obtained by direct smear from the mitral vegetation. The organism obtained by direct smear at autopsy was much longer and more slender than the shorter pleomorphic form obtained by culture on artificial mediums. A loopful of a twenty-four hour broth culture was inoculated on the chorioallantoic membrane of several 12 day old chick embryos. The bacilli multiplied rapidly and caused the death of the embryo within twenty-four hours. Smears made from the infected membrane showed long, slender granular rods in parallel arrangement, much more closely resembling those seen in the smear made directly from the valve (fig. 1 *E*).

Animal Inoculations.—Twenty-four hour broth cultures were inoculated subcutaneously in 2 cc. amounts into two guinea-pigs weighing 250 Gm., each, one of which had received 300 units of standard diphtheria antitoxin twenty-four hours previously. The unprotected guinea-pig died within forty-eight hours; the protected animal experienced no ill effects. Intraperitoneal inoculation of 0.25 cc. of a twenty-four hour broth culture caused death in an unprotected guinea-pig in three days. A control animal, protected twenty-four hours previously with 300 units of standard diphtheria antitoxin, survived.

Virulence tests, performed by making intracutaneous inoculations into guinea-pigs of 0.1 cc. amounts of undiluted culture and 1:5, 1:10, 1:20, 1:50 and 1:100 dilutions of a twenty-four hour broth culture gave positive reactions which went on to severe necrosis up to dilutions of 1:50. Control guinea-pigs, protected by 300 units of standard diphtheria antitoxin, showed no reaction.

The bacillus was cultured also in shallow flasks of nutrient broth over a period of seven days in order to determine whether or not a toxin was produced. The sterile Berkefeld V filtrate from a seven day old broth culture was injected in 0.1 cc. amounts intracutaneously into normal guinea-pigs and into guinea-pigs protected by a twenty-four hour injection of 300 units of diphtheria antitoxin. Positive reactions which progressed to severe necrosis were obtained in the normal animals with dilutions up to 1:20. The protected animals showed no reaction.

COMMENT

From all the available evidence it can be stated that this report adds another to the few cases of vegetative endocarditis caused by the B. diphtheriae recorded thus far in the literature. Morphologically and

culturally the organism isolated in this case belonged to the group Corynebacterium. Intracutaneous tests in guinea-pigs with a twenty-four hour broth culture and the filtrate of a seven day broth culture produced reactions typical of B. diphtheriae. Subcutaneous and intraperitoneal inoculations of the twenty-four hour broth culture caused death in an animal within three days. The administration of standard diphtheria antitoxin by the standard method protected guinea-pigs against the effect of the organism. These findings prove that the organism isolated from the mitral valve was a true strain of B. diphtheriae.

The portal of entry in this case most likely was the ulceration of the nasopharynx, which was caused by the prolonged packing necessitated by the continuous nosebleeds. The mitral valve, previously damaged by rheumatic disease, apparently provided a favorable lodging place for the organism, which gained entry to the blood stream from the ulcerated nasopharynx. From here embolic lesions were set up in the heart, intestines, kidneys, skin and mucous membranes. From the clinical course in this case it is obvious that the endocarditis was rapid and fulminating, lasting probably for only five days.

SUMMARY

A case of acute vegetative mitral endocarditis caused by B. diphtheriae is reported.

The bacillus isolated in pure culture from the heart blood, mitral vegetation and spleen at autopsy had the morphologic and cultural characteristics of B. diphtheriae. Inoculations into guinea-pigs demonstrated its toxin-producing capacity. Its toxic effect for guinea-pigs could be neutralized by the administration of standard diphtheria antitoxin.

CLINICAL SIGNIFICANCE OF BACTEREMIA IN PNEUMOCOCCIC PNEUMONIA

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This paper is concerned with an analysis of 1,586 cases of pneumonia associated with specific pneumococci of types I to XXXII (Cooper [1]) in which cultures of the blood were made during the acute stage of the disease or at autopsy. The purpose of this study is to correlate the incidence of bacteremia and the death rate with the more common factors known or supposed to influence the prognosis, including (1) the type of pneumococcus, (2) the age of the patient, (3) the sex, (4) the character of the pulmonary lesion, (5) the extent of the pulmonary involvement, (6) alcoholism, (7) preexisting diseases and conditions and (8) the leukocyte count. The effect of bacteremia on the incidence of septic complications and on the duration of the acute stage of the disease in cases of uncomplicated pneumonia is noted. The incidence of positive blood cultures on different days of the disease is correlated with the death rate. Variations in the results of multiple blood cultures during life and at autopsy are considered.

Few of these matters have received adequate consideration in the literature, except the comparative death rates for patients with pneumonia as regards the presence of bacteremia and occasional correlations with the types of pneumococcus. Most of the latter studies, furthermore, lose much of their value because the types were not adequately identified.

MATERIAL AND METHODS

The cases included in this analysis satisfy the following criteria: (1) the patients were 12 years of age or older and were admitted to the medical services of the Boston City Hospital between Nov. 1, 1929, and May 31, 1935; (2) definite

This study was aided, in part, by a grant given in memory of Francis Weld Peabody by the Ella Sachs Plotz Foundation.

From the Thorndike Memorial Laboratory, the Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.

1. Cooper, G.; Rosenstein, C.; Walter, A., and Peizer, L.: The Further Separation of Types Among the Pneumococci Hitherto Included in Group IV and the Development of Therapeutic Antisera for Those Types, J. Exper. Med. **55**: 531 (April) 1932.

evidence of pneumonia was demonstrated by physical signs, and except in occasional instances this was verified by roentgenograms or by postmortem examination or both; (3) pneumococci which agglutinated specifically in one of the antipneumococcus serums of types I to XXXII were isolated and identified from some source which suggested its etiologic relation to the pneumonia, namely, the sputum, blood or pulmonary or pleural fluid,[2] and (4) cultures of the blood were made on one or more occasions during the acute febrile stage of the illness and/or at necropsy. Patients having septic postpneumonic complications, such as empyema or pulmonary abscess, at the time of admission to the hospital were excluded.

A total of 2,448 blood cultures were made in the 1,586 cases. These included 382 cultures of heart blood taken postmortem, this being the only blood culture made in 210 of the cases. Cultures of venous blood were made at the bedside by introducing 5 or 10 cc. into 100 cc. of beef infusion broth (p_H 7.8), with 0.1 per cent dextrose added. Pour plates for colony counts were made in about half the cases, either by introducing 1 cc. (or smaller amounts when indicated) into 10 cc. of agar at the bedside or by using citrate as an anticoagulant and making the pour plates with 1 or 2 cc. of blood in the laboratory. At postmortem examination blood was obtained from the inferior vena cava within the pericardial cavity and was placed in broth and on blood agar plates. All pneumococci were isolated in pure culture and identified by morphologic and cultural characteristics, bile solubility and specific agglutination in antipneumococcus serums, either macroscopically or microscopically.[3] Serums for types I to XX only were available until May 1932 and for types I to XXXII thereafter. Throughout this study the cases are divided into two large groups: (1) the cases in which bacteremia was present—pneumococci were grown in culture of the blood *at some time* and (2) the cases in which bacteremia was not noted—*all* the blood cultures remained sterile. In most instances the cases in which specific antipneumococcus serums were used are considered separately.

RESULTS

The data will be presented chiefly in tabular form, supplemented by brief summaries and explanatory remarks.

Incidence of Bacteremia and the Mortality by Types (table 1).—Each of the specific serologic types of Cooper[1] was represented, although for a number of types there were too few cases to be significant and some of the latter included no cases in which bacteremia was present. For each type the death rate in the cases in which blood culture was positive was two or more times as high as in the cases in which the blood cultures were sterile, and for all types it averaged almost three times as high. For only a few of the types was the number of cases sufficiently large to warrant deductions with respect to variations in the incidence of bacteremia and in the mortality. From the data for these types, however, it appears that they may vary con-

2. Finland, M.: The Significance of Mixed Infections in Pneumococcic Pneumonia, J. A. M. A. **103**:1681 (Dec. 1) 1934.

3. (*a*) Avery, O. T.; Chickering, H. T.; Cole, R., and Dochez, A. R.: Acute Lobar Pneumonia: Prevention and Serum Treatment, Monograph 7, Rockefeller Institute for Medical Research, Oct. 16, 1917. (*b*) Sabin, A. B.: The Microscopic Agglutination Test in Pneumonia, J. Infect. Dis. **46**:469 (June) 1930.

siderably. Positive results of blood cultures were obtained in slightly more than a third of all the cases. The incidence of bacteremia varied widely in the cases of different types and was highest for the cases of pneumonia due to type II pneumococci. In the fatal cases the incidence of bacteremia was four times as great as for the nonfatal cases, and

TABLE 1.—*Incidence of Bacteremia and Mortality According to the Type of Pneumococcus*

		Blood Cultures										
		Positive			Negative			All Cases			Positive, %		
Type of Pneumococcus	Treatment with Serum	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	All Cases	Fatal Cases	Nonfatal Cases
I	S	69	25	36	123	15	12	192	40	21	36	63	29
I	..	123	104	86	142	37	26	265	141	53	47	74	15
II	S	31	20	65	47	6	13	78	26	33	40	77	21
II	..	62	47	76	45	14	31	107	61	57	58	77	33
III	..	118	116	98	144	58	40	262	174	66	45	67	2
IV	..	15	14	93	21	6	29	36	20	56	42	70	6
V	..	39	34	87	61	17	28	100	51	51	39	67	10
VI*	..	3	2	67	21	10	48	24	12	50	12	17	8
VII	S	2	1	50	8	0	0	10	1	10	20	100	11
VII	..	23	21	91	54	9	17	77	30	39	29	70	11
VIII	..	46	24	52	93	29	31	139	53	38	33	45	4
IX	..	4	3	75	21	6	29	25	9	36	16	33	26
X	..	6	5	83	30	14	47	36	19	53	17	26	6
XI	..	2	1	50	17	5	29	19	6	32	11	17	8
XII	..	10	8	80	18	3	17	28	11	39	36	73	12
XIII	..	3	2	67	12	4	33	15	6	40	20	33	11
XIV	..	9	8	89	24	8	33	33	16	48	27	50	6
XV	..	0	0	...	9	5	56	9	5	56	0	0	0
XVI	..	1	1	100	2	0	0	3	1	33	33	100	0
XVII	..	0	0	...	13	5	38	13	5	38	0	0	0
XVIII	..	7	7	100	26	11	42	33	18	55	21	39	0
XIX	..	1	1	100	15	2	13	16	3	19	6	33	0
XX	..	4	4	100	29	8	28	33	12	36	12	33	0
XXI	..	0	0	...	7	1	15	7	1	15	0	0	0
XXII	..	1	1	100	11	5	45	12	6	50	8	17	0
XXIII	..	1	1	100	1	1	100	2	2	100	50	50	..
XXIV	..	0	0	...	1	0	0	1	0	0	0	..	0
XXV	..	1	0	0	0	0	..	1	0	0	100	..	100
XXVII	..	1	1	100	0	0	..	1	1	100	100	100	..
XXVIII	..	0	0	...	2	1	50	2	1	50	0	0	0
XXIX	..	0	0	...	3	2	67	3	2	67	0	0	0
XXX	..	0	0	...	1	0	0	1	0	0	0	..	0
XXXI	..	0	0	...	2	0	0	2	0	0	0	..	0
XXXII	..	0	0	...	1	0	0	1	0	0	0	..	0
Serum treatment....		102	46	45.1	178	21	11.8	280	67	23.7	36.4	68.7	26.3
No serum treatment		480	405	84.4	826	261	31.6	1,306	666	51.4	36.8	60.8	11.7
Total.....		582	451	77.5	1,004	282	28.1	1,586	733	46.2	36.7	61.5	15.4

* This type includes types VIa, VIb and XXVI, designations which are no longer used.

this proportion was even higher when the cases in which serum treatment was given were excluded.

These findings are similar, in general, to those of Bullowa and Wilcox,[4] who noted that "in the main the percentages of invasiveness and mortality are approximately the same." The higher death rates consistently recorded in the present report were probably due to the selection of cases, since we excluded all nonfatal cases in which no

4. Bullowa, J. G. M., and Wilcox, C.: Incidence of Bacteremia in the Pneumonias and Its Relation to Mortality, Arch. Int. Med. **55**:558 (April) 1935.

blood cultures were made before the crisis and included many cases of bronchopneumonia and also cases in which the type was first determined at necropsy. The age distribution in this series also may have contributed to the higher mortality, as children were excluded, and

TABLE 2.—*Incidence of Bacteremia and Mortality According to Age and Sex*

	Blood Cultures									
	Positive			Negative			All Cases			
Age Group	Number of Cases	Fatal Cases	Fatal Cases, %	Number of Cases	Fatal Cases	Fatal Cases, %	Number of Cases	Fatal Cases	Fatal Cases, %	Positive, %
12-19	22	6	27	124	10	8	146	16	11.0	15.1
20-29	58	32	55	177	22	12	235	54	23.0	24.7
30-39	88	53	60	209	27	13	297	80	26.9	29.6
40-49	137	107	78	182	46	25	319	153	48.0	42.9
50-59	106	91	86	172	81	47	278	172	61.9	39.1
60-69	107	98	92	72	39	54	179	137	76.5	59.8
70 +	64	64	100	68	57	84	132	121	91.7	48.5
Total.....	582	451	77.5	1,004	282	28.1	1,586	733	46.2	36.7
Males.....	408	314	77.0	705	185	26.2	1,113	499	44.8	36.7
Females.....	174	127	78.7	299	97	32.4	473	234	49.5	36.6

TABLE 3.—*Bacteremia in the More Common Types According to Age*

	12 to 29 Years Blood Cultures							30 to 49 Years Blood Cultures							50 Years or More Blood Cultures						
	Positive				Negative			Positive				Negative			Positive				Negative		
Type of Pneumococcus	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %
I*	23	3	13	29	56	3	5	32	14	44	38	52	6	12	14	8	57	48	15	6	40
I	14	8	57	20	44	3	7	51	40	78	45	63	10	16	58	56	97	62	35	24	69
II*	8	5	62	26	23	0	0	15	9	60	48	16	3	19	8	6	75	50	8	3	38
II	8	4	50	32	17	4	24	27	19	70	61	17	3	18	27	24	89	71	11	7	64
III	5	5	100	20	20	1	5	27	26	96	34	52	12	23	86	85	99	54	72	45	63
IV	2	2	100	29	5	0	0	6	5	83	37	10	2	20	7	7	100	54	6	4	67
V	7	4	57	29	17	2	12	13	12	92	32	27	6	22	19	18	95	53	17	9	53
VI	0	0	...	0	7	4	57	1	0	0	14	6	1	17	2	2	100	20	8	5	63
VII	1	0	0	4	22	1	5	13	12	92	37	22	1	5	11	10	91	38	18	7	39
VIII	3	1	33	15	17	4	24	25	9	36	37	42	9	21	18	14	78	35	34	16	47
IX	0	0	...	0	4	0	0	0	0	...	0	7	3	43	4	3	75	29	10	3	30
X	0	0	...	0	5	1	20	1	1	100	11	8	2	25	5	4	80	23	17	11	65
XII	1	1	100	17	5	0	0	3	3	100	25	9	2	22	6	4	67	60	4	1	25
XIV	3	2	67	30	7	0	0	2	2	100	18	9	1	11	4	4	100	33	8	7	88
XVIII	1	1	100	8	11	3	27	3	3	100	30	7	2	29	3	3	100	27	8	6	75
XX	1	1	100	20	4	0	0	1	1	100	8	12	2	17	2	2	100	13	13	6	46
Others	3	1	33	8	37	6	16	5	4	80	14	32	8	25	3	3	100	10	28	17	61
Total	80	38	48	21	301	32	11	225	160	71	37	391	73	19	277	253	91	47	312	177	57

* Treatment with serum.

possibly, also, there was a larger percentage of older patients than usual.

Age (tables 2 and 3; chart 1).—The mortality rate increased progressively with the ages of the patients, this being true for patients

both with and without bacteremia. It was also true, in general, of the patients with each type of pneumonia. The incidence of bacteremia in each age group paralleled the mortality through the fifth decade, after which the mortality continued to increase steadily, whereas the incidence of bacteremia showed a relative decline up to the eighth decade and an absolute decline later. This is seen graphically by comparing the heights of the solid and of the adjacent striped bars in chart 1. The incidence of bacteremia in both the fatal and the nonfatal cases was relatively lower in the aged patients (70 years and over) and in adolescent children (from 12 to 19 years) than in the other age groups.

Sex (table 2).—The incidence of bacteremia and the death rates were very similar for males and females.

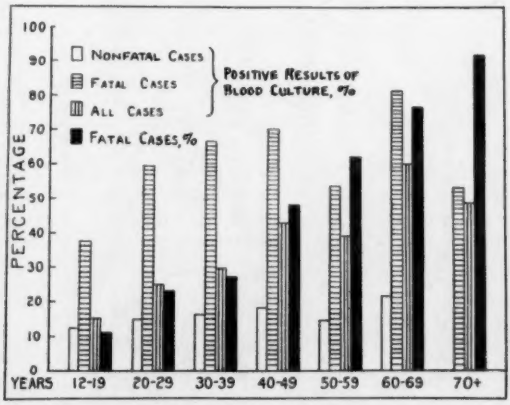

Chart 1.—Comparison of the incidence of bacteremia and the mortality for various age groups.

Lobar Pneumonia and Bronchopneumonia (table 4).—It was pointed out by Sutliff and Finland [5] that for all types of pneumococci except type III the mortality in cases of bronchopneumonia is considerably higher than in cases of lobar pneumonia due to the same type of pneumococcus. The greater mortality in the cases of bronchopneumonia (better termed "atypical" pneumonia [6]) was here found in each type, both with and without bacteremia. Bacteremia, however, was less frequent for most types in the cases of atypical pneumonia, indicating

5. Sutliff, W. D., and Finland, M.: The Significance of the Newly Classified Types of Pnuemococci in Disease: Types IV to XX Inclusive, J. A. M. A. **101**: 1289 (Oct. 21) 1933.

6. Cole, R.: Acute Pulmonary Infections, in De Lamar Lectures, 1927-1928, Baltimore, Williams & Wilkins Company, 1928.

that the high mortality in such cases is due to the frequency with which this kind of pneumonia occurs as a complication or as a terminal event in the course of other serious conditions.[5]

Extent of Pulmonary Involvement (table 5).—The death rate was higher in the cases in which the greater amount of lung was involved. This was true regardless of whether or not bacteremia was noted, and also, in a general way, within the different age groups. Bacteremia was considerably more frequent in the cases in which two or more lobes

TABLE 4.—*Incidence of Bacteremia and Mortality in Cases of Lobar Pneumonia and in Bronchopneumonia*

Type of Pneumococcus Treatment with Serum		Lobar Pneumonia Blood Cultures							Bronchopneumonia Blood Cultures						
		Positive				Negative			Positive				Negative		
		No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %
I	S	69	25	36	36	123	15	12	0	..	...	...	0	..	..
I	..	114	93	82	45	137	32	23	9	9	100	64	5	..	100
II	S	31	20	65	40	47	6	13	0	..	...	...	0	0	..
II	..	61	46	75	58	42	11	26	1	1	100	25	3	3	100
III	..	100	98	98	47	115	43	37	18	18	100	38	29	15	52
IV	..	11	10	91	41	16	3	19	4	4	100	44	5	3	60
V	..	32	27	84	38	52	12	23	7	7	100	44	9	5	56
VI	..	2	2	100	11	16	7	44	1	0	0	17	5	3	60
VII	S	1	0	0	11	8	0	0	1	1	100	100	0	0	..
VII	..	20	18	90	29	49	5	10	3	3	100	38	5	4	80
VIII	..	38	17	45	38	62	11	18	8	7	87	21	31	18	58
IX	..	3	2	67	18	14	3	21	1	1	100	13	7	3	43
X	..	3	2	67	15	17	5	29	3	3	100	19	13	9	69
XII	..	8	6	75	32	17	3	18	2	2	100	67	1	0	0
XIV	..	9	8	89	37	15	2	13	0	..	...	0	9	6	67
XVIII	..	4	4	100	18	18	5	28	3	3	100	27	8	6	75
XX	..	0	..	..	0	16	3	19	4	4	100	24	13	5	38
Others	..	7	5	71	10	64	14	22	4	3	75	11	33	17	51
Serum treatment		101	45	44.5	35	178	21	11.8	1	1	100.0	..	0	0	..
No serum treatment		412	340	83.2	30	650	159	24.4	68	67	98.5	28	176	102	58.0
Total		513	385	75.0	38	828	180	21.7	69	66	95.0	28	176	102	58.0

were involved than in the cases in which the disease was limited to a single lobe. When three lobes were involved the incidence of bacteremia was somewhat higher than when two lobes were involved. For any given amount of involvement there was a higher incidence of bacteremia in the older age groups, but this difference was especially marked in the cases in which a single lobe was affected. Involvement of two or three lobes was more frequent in the older age groups (table 6).

Alcoholism (table 7).—There was little difference in the incidence of bacteremia or in the death rates among those patients who said they

had not used alcohol and those who were habitual drinkers, including even those who were acutely intoxicated from alcohol or had delirium tremens at the time of admission to the hospital. The younger alcoholic addicts, however, showed a higher incidence of bacteremia and a higher

TABLE 5.—*Incidence of Bacteremia and Mortality in Relation to the Number of Lobes of the Lungs Involved*

		Blood Cultures											
		Positive			Negative			Positive			Negative		
Age Group	Treatment*	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %
		One Lobe Involved							Two Lobes Involved						
12-29	Serum	16	2	13	19	68	1	1	11	3	27	52	10	2	20
	No serum	16	6	38	11	132	9	7	18	13	72	33	36	7	19
30-49	Serum	18	6	33	24	56	3	5	14	6	43	56	11	5	45
	No serum	60	41	68	26	173	26	15	48	39	81	40	72	16	22
50 and over	Serum	9	6	67	37	15	5	33	8	5	63	62	5	1	20
	No serum	87	79	91	49	90	31	34	70	67	96	55	58	36	62
All ages	Serum	43	14	33	24	139	9	6	33	14	42	56	26	8	31
	No serum	163	126	77	29	395	66	17	136	119	87	45	166	59	36
		Three Lobes Involved							Four or Five Lobes Involved						
12-29	Serum	4	2	50	57	3	0	0	1	1	100	33	2	0	0
	No serum	5	3	60	22	18	3	17	3	2	67	30	7	3	43
30-49	Serum	12	8	67	75	4	0	0	3	3	100	75	1	1	100
	No serum	42	33	78	60	28	4	14	8	6	75	57	6	4	67
50 and over	Serum	4	2	50	67	2	2	100	1	1	100	50	1	1	100
	No serum	45	41	91	65	24	14	58	10	10	100	62	6	6	100
All ages	Serum	20	12	60	69	9	2	22	5	5	100	56	4	2	50
	No serum	92	77	84	57	70	21	30	21	18	86	52	19	13	68

* Types I, II and VII serums were used.

TABLE 6.—*Extent of the Pulmonary Lesion in Different Age Groups*

Number of Lobes Involved	12 to 29 Years		30 to 49 Years		50 Years or More	
	No. of Cases	Incidence, %	No. of Cases	Incidence, %	No. of Cases	Incidence, %
1	232	66.3	307	55.2	201	46.2
2	75	21.4	145	26.1	141	32.4
3	30	8.6	86	15.5	75	17.2
4 or 5	13	3.7	18	3.2	18	4.1
Total	350	100.0	556	100.0	435	100.0

mortality than the abstainers in the same age group, but there was only a small number of such young imbibers. Only 9 per cent of all the alcoholic patients were less than 30 years of age, as compared with 32 per cent of the abstainers.

Preexisting Systemic Diseases and Other Complicating Conditions (table 8).—There was a significantly greater incidence of bacteremia

TABLE 7.—*Influence of Alcoholism on Incidence of Bacteremia and on Death Rates*

Age Group	Treatment	Blood Cultures: Positive — No. of Cases	Positive — Fatal Cases	Positive — Fatal Cases, %	Positive, %	Negative — No. of Cases	Negative — Fatal Cases	Negative — Fatal Cases, %	Positive — No. of Cases	Positive — Fatal Cases	Positive — Fatal Cases, %	Positive, %	Negative — No. of Cases	Negative — Fatal Cases	Negative — Fatal Cases, %
		Abstainers and Infrequent Drinkers							Moderate Chronic Drinkers						
12-29	Serum	22	6	27	26	62	2	3	2	0	0	29	5	0	0
	No serum	25	13	50	18	120	15	13	2	2	100	22	7	1	14
30-49	Serum	26	11	42	40	39	1	3	5	4	80	33	10	3	30
	No serum	64	47	73	32	134	21	16	23	17	74	32	50	10	20
50 and over	Serum	8	5	62	38	13	5	39	7	5	71	64	4	1	25
	No serum	102	95	93	51	100	61	61	22	19	81	42	30	16	53
All ages	Serum	56	22	39	33	114	8	7	14	9	64	42	19	4	21
	No serum	192	155	81	35	354	97	27	47	38	81	35	87	27	31
		Acutely Intoxicated and/or with Delirium Tremens							Alcoholic History Not Determined						
12-29	Serum	2	0	0	40	3	0	0	6	2	33	32	13	1	8
	No serum	3	3	100	38	5	1	20	17	12	71	17	85	12	14
30-49	Serum	6	3	50	38	10	1	10	11	6	54	46	13	4	31
	No serum	32	26	81	48	35	8	23	58	46	79	37	100	25	25
50 and over	Serum	3	2	67	43	4	1	25	4	2	50	67	2	2	100
	No serum	19	18	95	43	25	8	32	112	107	95	45	135	83	61
All ages	Serum	11	5	45	39	17	2	12	21	10	48	43	28	7	25
	No serum	54	47	87	46	65	17	26	187	165	88	37	320	120	38

TABLE 8.—*Effect of Preexisting Complicating Conditions and Systemic Diseases on the Incidence of Bacteremia and on the Mortality*

Complicating Condition	Positive Blood Cultures: Serum Treatment — No. of Cases	Serum Treatment — Fatal Cases	Serum Treatment — Fatal Cases, %	Positive Blood Cultures: No Serum Treatment — No. of Cases	No Serum Treatment — Fatal Cases	No Serum Treatment — Fatal Cases, %	Positive Blood Cultures, %: Serum Treatment	Positive Blood Cultures, %: No Serum Treatment	Negative Blood Cultures: Serum Treatment — No. of Cases	Serum Treatment — Fatal Cases	Serum Treatment — Fatal Cases, %	Negative Blood Cultures: No Serum Treatment — No. of Cases	No Serum Treatment — Fatal Cases	No Serum Treatment — Fatal Cases, %
Cardiac disease	7	7	100	89	85	96	54	50	6	2	33	89	61	68
Pregnancy and the puerperium	7	1	14	11	8	73	54	48	6	1	17	12	5	42
Delirium tremens	7	2	29	20	20	100	70	58	3	1	33	18	7	39
Postoperative	1	0	0	10	7	70	100	36	..	..	..	18	9	50
Cerebral accident	..	..	...	9	9	100	...	47	..	..	..	10	8	80
Carcinoma	..	..	...	9	9	100	...	56	..	..	..	7	6	86
Nephritis and uremia	1	1	100	6	6	100	100	60	..	..	..	4	4	100
Leukemia and pernicious anemia	..	..	...	6	5	83	...	86	..	..	..	1	0	0
Tuberculosis (pulmonary)	..	..	...	7	5	71	0	50	1	1	100	7	1	14
Asthma (bronchial)	1	0	0	5	4	80	20	31	4	1	25	11	3	27
Cirrhosis of liver	1	1	100	4	4	100	100	57	..	..	..	3	2	67
Diabetes	..	..	...	5	5	100	...	38	..	..	..	8	5	63
Diabetic coma	..	..	...	1	1	100	...	17	..	..	..	5	4	80
Acute infectious diseases (other than pneumococcic)	..	..	...	3	3	100	...	18	..	..	..	14	8	57
Miscellaneous (trauma, morphine addiction, neuritis, embolus, epilepsy, scurvy, ulcer, hemachromatosis, bronchiectasis, sickle cell anemia, gangrene, decubitus, iodine poisoning, periarteritis nodosa, burns)	2	2	100	10	8	80	50	20	2	1	50	25	14	56
With previous disease	27	14	52	195	179	92	55	46	22	7	32	232	137	59
Without complicating disease	75	32	43	285	226	79	82	32	156	14	9	594	124	21

TABLE 9.—*Relation of the Leukocyte Count to Incidence of Bacteremia and to Mortality*

Leukocyte* Count, Thousands	Blood Cultures						
	Positive				Negative		
	Number of Cases	Fatal Cases	Fatal Cases, %	Total Cases, %	Number of Cases	Fatal Cases	Fatal Cases, %
Less than 5	33	32	97	69	15	8	53
5 to 9	58	55	95	40	87	35	40
10 to 14	108	87	81	33	223	45	20
15 to 24	160	106	66	31	364	93	26
25 to 34	57	32	56	33	113	26	23
35 and over	27	22	82	40	41	12	29
Not made	139	117	84	46	161	63	39
Total	582	451	78	37	1,004	282	28

* The mean of the counts taken during the acute febrile period of pneumonia was usually taken. If the fluctuations were wide, the highest or lowest count alone was included when that seemed more significant than the mean.

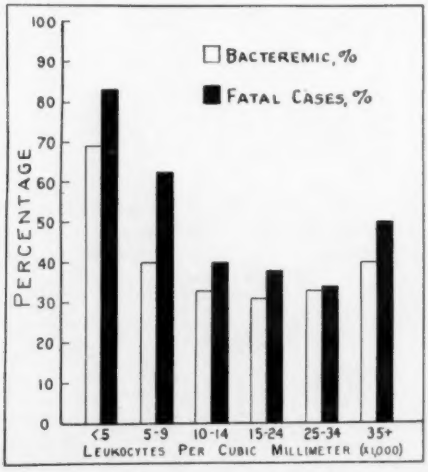

Chart 2.—Comparison of the incidence of bacteremia and the mortality for various levels of the leukocyte count.

in the cases of pneumonia which occurred in the course of other serious conditions or diseases (so-called secondary pneumonia) than in the cases of primary pneumonia. The death rate in the cases of secondary pneumonia was higher both when positive and when negative blood cultures were obtained, but the difference was most striking in the latter cases.

The Leukocyte Count (table 9 and chart 2).—The differences in the mortality rate at different leukocyte levels varied in the same manner as the incidence of bacteremia. Bacteremia was most frequent in the cases in which the leukocyte count was below 10,000. It was higher

in the cases in which the count was 35,000 or more than in those in which it was between 10,000 and 34,000. The mortality in all the cases corresponded to the incidence of bacteremia, except in the cases in which the count was less than 10,000, in which there was a relatively higher death rate. The lower counts were somewhat more frequent in the cases of bacteremia.

Postpneumonic Complications (table 10).—Bacteremia was more than twice as frequent in the cases in which there were postpneumonic

TABLE 10.—*Relation of Bacteremia to the Incidence and Mortality of Postpneumonic Complications*

	Blood Cultures												
	Positive							Negative					
	Serum Treatment			No Serum Treatment			Total Cases, %	Serum Treatment			No Serum Treatment		
	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	Serum Treatment	No Serum Treatment	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %
Empyema	16	5	31	61	55	90	73	65	6	2	33	33	16	49
Sterile pleural effusion	5	3	60	8	4	50	36	26	9	1	11	23	3	13
Pulmonary abscess	2	2	100	24	24	100	50	63	2	2	100	14	11	79
Pericarditis	..	..	...	21	21	100	0	78	1	1	100	6	6	100
Endocarditis	3	3	100	10	10	100	100	83	..	..	...	3	3	100
Peritonitis	..	..	...	1	1	100	...	100	..	..	...	..	..	...
Meningitis	2	2	100	16	16	100	100	94	..	..	...	1	1	100
Parotitis	1	0	0	1	1	100	50	50	1	1	100	1	0	0
Arthritis	1	0	0	4	3	75	100	100	..	..	...	..	..	...
Subcutaneous abscess	2	0	0	3	2	67	100	60	..	..	...	3	1	50
Thrombophlebitis	1	0	0	3	2	67	50	60	1	0	0	3	0	0
Otitis media	..	..	...	3	3	100	0	25	3	0	0	9	3	33
Conjunctivitis	..	..	...	2	1	50	...	100	..	..	...	..	..	...
Streptococcic infection	4	2	50	1	0	0	100	11	..	..	...	8	5	63
Relapse	..	..	...	1	0	0	...	33	..	..	...	2	0	0
With complications*	33	13	39	126	113	90	61	56	21	5	24	98	41	42
Without complications	69	33	48	354	292	82	31	33	157	16	10	728	220	30
With complications, %	32	..	..	26	..	..	..	..	12	..	..	12	..	..

* Cases of multiple complications are included with each of the complications but only once in the totals.

complications as in those in which there were none. Conversely, the complications were more than twice as frequent in the cases of bacteremia as in the cases in which bacteremia was not noted. The death rate in the cases in which there were postpneumonic complications was higher than in the cases in which pneumonia terminated without such complications, except for the cases of bacteremia in which serum treatment was given. The situation was different in the cases in which the pleural effusion was sterile, the incidence of bacteremia and the death rates in these cases being similar to those in the cases in which there were no "septic" complications.

TABLE 11.—*Duration of the Acute Illness*

Positive blood cultures	Duration of the Acute Illness, Days															Day of Onset or Termination Indefinite	Total
	1	2	3	4	5	6	7	8	9	10	11	12	13 to 15	16 to 20	20 and Over		
Serum																	
Number of cases...	0	1	3	13	14	15	12	10	5	3	5	2	5	1	4	9	102
Fatal cases.....	..	1	1	2	8	5	3	8	3	2	3	1	3	0	4	2	46
Fatal cases, %.....	..	100	33	15	57	33	25	80	60	67	60	50	60	0	100	22	45
No serum																	
Number of cases...	2	5	9	25	21	37	51	42	48	39	27	18	22	19	19	96	480
Fatal cases.....	2	4	6	22	19	26	45	33	35	30	25	15	22	17	18	86	405
Fatal cases, %.....	100	80	67	88	91	70	88	79	73	77	93	83	100	89	95	90	84
Negative blood cultures																	
Serum																	
Number of cases...	1	2	12	39	43	30	18	12	5	1	3	2	7	0	2	1	178
Fatal cases.....	0	0	1	1	3	1	5	3	2	0	1	0	2	..	2	0	21
Fatal cases, %.....	0	0	8	3	7	3	28	25	40	0	33	0	29	..	100	0	12
No serum																	
Number of cases...	0	9	20	41	67	71	80	97	83	42	36	27	49	25	23	147	826
Fatal cases.....	..	4	4	16	12	15	23	17	18	8	13	10	14	9	19	79	261
Fatal cases, %.....	..	44	20	39	18	21	26	18	22	19	36	37	29	36	83	54	32
Serum treatment.....	1	3	15	52	57	45	30	22	10	4	8	4	12	1	6	10	280
No serum treatment...	2	14	29	66	88	108	140	139	131	81	63	45	71	44	42	243	1,306
Total.....	3	17	44	118	145	153	170	161	141	85	71	49	83	45	48	253	1,586

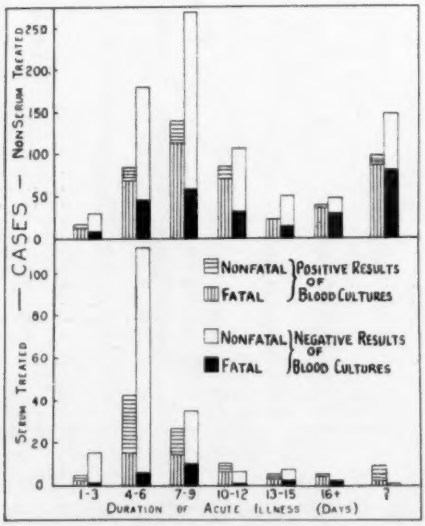

Chart 3.—Comparison of the duration of the acute illness, the mortality, the incidence of bacteremia and the type of treatment for various age groups. The height of the columns represents the number of cases.

Duration of the Acute Disease (table 11 and chart 3).—In the cases in which no specific serum was given the termination of the acute disease by crisis, lysis or death occurred most frequently between the seventh and the ninth day. This was true regardless of the presence of bacteremia. In the cases in which serum was administered recovery was usually between the fourth and the sixth day, regardless of whether

positive or negative blood cultures were obtained. Fatalities in the cases in which specific treatment for bacteremia was given occurred with equal frequency between the fourth and the sixth day and between the seventh and the ninth day, whereas the fatalities in the cases in which bacteremia was not present and in which serum was administered were most frequent during the latter interval. In the cases of pneumonia either with or without bacteremia in which serum was not given the death rates were higher in cases in which the disease terminated after the tenth day than in those in which the acute disease ended earlier. In the cases in which serum was given the mortality was higher when the disease ended after the seventh day when the blood

TABLE 12.—*Incidence of Bacteremia and Mortality on Each Day of Illness* *

	Serum Treatment Blood Cultures							No Serum Treatment Blood Cultures						
	Positive				Negative			Positive				Negative		
Day of Illness	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %	No. of Cases	Fatal Cases	Fatal Cases, %	Positive, %	No. of Cases	Fatal Cases	Fatal Cases, %
1 and 2.....	19	6	31	34	37	5	14	22	12	54	19	92	31	33
3.....	32	14	44	28	82	5	6	33	23	70	22	118	26	22
4.....	23	10	43	24	74	11	15	57	42	74	32	118	21	18
5.....	21	9	43	24	67	12	18	54	40	74	28	141	29	25
6.....	11	6	54	19	45	10	22	47	35	74	30	147	40	27
7.....	13	10	77	31	28	7	25	58	46	78	37	100	21	21
8.....	7	3	43	33	14	4	29	55	46	83	37	94	21	22
9.....	4	2	50	25	12	4	33	39	36	92	42	48	12	25
10.....	2	1	50	20	8	5	63	24	22	91	35	45	14	31
After 10.....	11	7	64	41	16	6	38	46	40	87	37	80	31	39
Not determined...	2	2	100	50	2	2	100	39	36	92	40	59	30	41

* Blood cultures taken after recovery by crisis or lysis are not included.

cultures were positive or after the sixth day when the blood cultures were sterile.

Blood Cultures on Various Days of Illness (table 12).—For the cases in which positive blood cultures were obtained there was a higher mortality than for the cases in which negative cultures were obtained, irrespective of the day when the cultures were taken. The incidence of bacteremia when serum treatment was withheld increased moderately as the disease advanced, and this was accompanied with some increase in the death rate, particularly in the cases of bacteremia.

Variations in the Results of Blood Cultures in the Same Case (table 13).—Sterilization of the blood stream without apparent improvement of the acute symptoms or in spite of a progression of the pulmonary lesion occurred more frequently after serum therapy was given than in cases in which no serum was administered. The mortality in such

cases, both with and without serum treatment, was lower than for the entire group of cases of pneumonia with bacteremia (table 1); when serum was not given, however, the mortality was still high. The explanation for the lack of adequate improvement and for the occasional initial appearance of a positive blood culture after the beginning of serum therapy may rest on the inadequacy or lateness of the treatment.[7] The death rate in the cases in which positive blood cultures were

TABLE 13.—*Outcome in Cases in Which Both Positive and Negative Blood Cultures Were Obtained*

		Serum Treatment	No Serum Treatment	Total Number of Cases
Positive cultures, followed by negative cultures during acute illness or at autopsy	Number of cases	48	43	91
	Fatal cases	9	26	35
	Fatal cases, %	19	60	38
Negative cultures, followed by positive cultures during life or at autopsy	Number of cases	4	47	51
	Fatal cases	3	40	43
	Fatal cases, %	75	85	84

TABLE 14.—*Results of Cultures of Heart Blood at Autopsy*

		Postmortem Blood Cultures					
		No Serum Treatment			Serum Treatment		
Cultures Made During Life	Total Number of Cases	Positive	Negative	Positive, %	Positive	Negative	Positive, %
None	209	114	94	55	1	0	100
Positive	91	62	18	78	8	3	73
Negative	55	18	31	37	1	5	17
Positive and later negative	4	1	1	50	0	2	0
Negative and later positive	10	6	2	75	1	1	50
Negative, positive and later negative	1	0	0	..	1	0	0
Successively positive, negative, positive and negative	3	0	1	0	0	2	0
Positive for hemolytic streptococcus*	8						
Positive for Friedländer's bacillus†	1						
Total	382	201	147	58	12	13	48

* Antemortem cultures: 5 negative and 2 that were first negative and then positive (for pneumococcus). In 1 case no antemortem culture was made.
† Positive for Friedländer's bacillus during life.

obtained after the cultures had been sterile was the same as for all the cases of bacteremia.

Postmortem Blood Cultures (table 14).—The incidence of positive cultures of heart blood, made usually from two to eighteen hours post mortem, was similar to that found in all the fatal cases (table 1). Positive cultures were obtained for heart blood in over a third of the

7. Finland, M., and Dowling, H. F.: The Dose of Antibody Effective in the Treatment of Pneumococcus Type II Pneumonia, Am. J. M. Sc. **191**:658 (May) 1936. Finland, M.: Adequate Dosage in the Specific Serum Treatment of Pneumococcus Type I Pneumonia, ibid. **192**:849 (Dec.) 1936.

cases in which such cultures were made after sterile cultures had been obtained during life.

Bacteremia and Multiple Types of Pneumococci (table 15).—Positive blood cultures may be of great assistance in determining the etiologically significant organism in cases of pneumonia with "mixed infections." Type I pneumococcus invaded the blood stream in all 9 cases of bacteremia in which this type occurred along with other types of pneumococci. Type III pneumococci, on the other hand, invaded the blood in only 6 of the 9 cases of bacteremia in which this type and others were involved. Type X occurred in 3 cases and type XX in 2 cases in which other types of pneumococci were cultured from the

TABLE 15.—*Multiple Types of Pneumococcus in Cases of Bacteremia*

Case*	Termination†	Type of Pneumococcus				
		Sputum	Blood Culture During Life	Pleural Fluid	Lung (Autopsy)	Heart Blood (Autopsy)
1	D	I, III	I	..	..	..
2	D	X	I	..	I	0
3	D	I, III	I, III	..	I, III	..
4	D	XXX	I	..	I	0
5	D	VI	..	..	I	I
6	D	I, X	I	..	..	..
7	D	XX	..	..	..	I
8	D	I, XXIV	I	..	I	I
9	D	I, II	I	..	I	I
10	D	XX	II	..	..	..
11	D	III, VIII	III	..	..	III
12	D	III, XXI	III	..	..	..
13	D	III	III	..	III, XXIV	III
14	D	III, XXIII	III	..	..	..
15	D	VIII	III	..	..	..
16	D	..	IV	..	IV, XIII	IV
17	D	V	..	V	V, X	V
18	R	III, XI	XI	..	..	..
19	D	III, XIV	XIV	..	XIV	XIV
20	R	X, XXXI	XXV	..	..	..

* In cases 1 to 3 serum treatment was given.
† R indicates recovered; D, died.

blood. It cannot always be determined, however, whether such findings are the result only of the lack of invasiveness of certain types or whether they indicate that these types are not related to the pulmonary infection. Other methods may be invoked and may help in such cases or in cases in which sterile cultures are obtained.[2] In only 1 of our 20 cases were two types identified in the blood. In this case type I and type III pneumococci were found. It is possible and likely that other instances of invasion of the blood stream with more than one type of pneumococcus have been missed.

Quantitative Blood Cultures.—Fluctuations from day to day between positive and negative blood cultures for the same patient, even with specific serum therapy, have already been noted. Such variation may depend, in general, on the degree of bacteremia, that is, the number of pneumococci per cubic centimeter of blood, or possibly on the methods

used in taking the cultures. In a number of cases, however, the blood exhibited a remarkable ability to sterilize itself even without therapy and occasionally even after a heavy invasion. For the bacteremic patients who recovered the colony count was usually below 10 per cubic centimeter of blood.[8] The prognosis was usually unfavorable when the number of colonies increased in successive blood cultures,[9] but this was not invariably true, as will be seen from some of the cases to be cited here.

The heaviest invasion encountered in a fatal case was estimated at 35,000 colonies of type II pneumococci per cubic centimeter of blood. This was obtained in a 53 year old patient with cirrhosis of the liver and pneumonia involving two lobes. The largest number of colonies per cubic centimeter grown from the blood of a patient who recovered without serum therapy was 1,700. This was a case of type V pneumonia and will be described herewith. A few instances in which the blood became sterile after heavy invasion and without specific therapy will be noted briefly.

A man aged 62 years with a single lobe of one lung consolidated showed 20 colonies of type I pneumococcus per cubic centimeter of blood on the eighth day of his illness. Daily cultures thereafter showed 70, 200, 80, 50, 12, 29 and 2 colonies per cubic centimeter of blood respectively. The cultures taken from the fifteenth to the nineteenth day, inclusive, were all sterile. The patient died on the twenty-third day of his illness, and the culture of the heart blood at autopsy was sterile.

Recovery occurred in a case of pneumonia due to type I pneumococci with 225 colonies per cubic centimeter of blood. The patient was a man 34 years old who showed acute alcoholic intoxication at the time of his admission to the hospital. Blood cultures showed 60 colonies per cubic centimeter on the fourth day, 225 colonies on the fifth, 21 colonies on the eighth, a positive culture in broth but no growth in the plates on the twelfth day and sterile cultures on the fifteenth, sixteenth and nineteenth days. His convalescence was complicated by septic arthritis due to type I pneumococcus, but eventually he made a complete recovery.

On the fifth day of illness a 32 year old man who showed acute alcoholic intoxication at the time of his admission to the hospital had 250 colonies of type II pneumococcus per cubic centimeter of blood. The entire right lung was consolidated. No specific therapy was given. On the seventh day the pour plates showed 17 colonies per cubic centimeter of blood; and on the ninth day the cultures were sterile and lysis began. The patient had a small empyema and conjunctivitis, from both of which type II pneumococci were cultured. Both these complications cleared rapidly and completely, the former after a single thoracentesis and the latter after local irrigations.

A woman of 27 had pneumonia due to type V pneumococci after a miscarriage. On the second day of the pneumonia a culture showed 50 colonies per cubic

8. Cole, R.: Acute Lobar Pneumonia, in Nelson Loose-Leaf Living Medicine, New York, Thomas Nelson & Sons, 1920, vol. 1, p. 248.

9. Koch, K. R.: The Prognostic Value of Repeated Blood Cultures in Pneumococcus Lobar Pneumonia, Am. J. M. Sc. **184**:364 (Sept.) 1932.

centimeter of blood. On the third day there were 5 colonies and on the fourth day 1,700 colonies per cubic centimeter of blood. On the fourth day dilation and curettage, with removal of retained placental fragments were carried out. Blood cultures taken on each of the next two days were sterile. Recovery by lysis began on the ninth day and was followed by an uneventful convalescence.

A man aged 21 showed 65 colonies of type VII pneumococci per cubic centimeter of blood on the twenty-sixth day of illness. Blood culture on the thirty-first day was sterile, but the patient died of empyema two days later.

Résumé of the Data Relating to Specific Serum Therapy.—During the period covered by this study concentrated specific antipneumococcus serum was used in the treatment in a large proportion of the cases of type I and type II pneumonia. The results of this therapy are dealt with in greater detail elsewhere.[10] A small number of patients with type VII pneumonia were treated with specific serum during the latter part of this study. In the accompanying tables all of these cases in which specific treatment was given and in which blood cultures were made are included and for the most part listed separately, in order to indicate the differences in death rate when compared with the cases in which specific serum treatment was not given. It is well to review these findings briefly.

The incidence of bacteremia was the same for all the serum-treated patients as for the nonserum-treated patients. The survivals after serum therapy included a much larger percentage of the cases of bacteremia than was found for the cases in which recovery was obtained without serum treatment (table 1).

For the cases in which bacteremia was not present, the differences in mortality were equally striking in each of the types in which serum was used. A similar difference was noted in the cases of type I pneumonia in which blood culture was positive, but the difference was not nearly as striking in the cases of type II pneumonia (table 1).

The young and the old patients, the bacteremic and the nonbacteremic, all shared in varying degrees in the reduction in mortality from serum therapy (table 3). There are no data available concerning serum therapy in cases of bronchopneumonia (table 4).

In general, the death rates were lower among the serum-treated patients, irrespective of the number of lobes of the lungs involved, both in those with and in those without bacteremia (table 5).

Serum-treated alcoholic patients also showed a lower mortality than nonserum-treated patients with similar alcoholic habits, irrespective of

10. Sutliff, W. D., and Finland, M.: Type I Pneumococcic Infections with Especial Reference to Specific Serum Treatment, New England J. Med. **210**: 237 (Feb. 1) 1934. Finland, M., and Sutliff, W. D.: The Specific Serum Treatment of Pneumococcus Type II Pneumonia, J. A. M. A. **100**:560 (Feb. 25) 1933. Finland and Dowling.[7]

age and the "degree" of alcoholism. This was true for both the bacteremic and the nonbacteremic patients (table 7).

Patients with significant preexisting systemic diseases likewise showed a lower mortality if treated with serum. The differences in the death rates were similar to those found among patients without these complicating conditions (table 8).

Septic complications occurred with equal frequency among serum-treated and nonserum-treated patients. This was true for both bacteremic and nonbacteremic patients (table 10). Large sterile effusions occurred in a higher percentage of serum-treated (5 per cent) than in untreated patients (3 per cent).

Recovery by crisis or lysis occurred earlier in the serum-treated patients, both bacteremic and nonbacteremic. No striking difference between serum-treated and nonserum-treated patients was observed with regard to the duration of the disease in the fatal cases (table 11; fig. 3).

The incidence of bacteremia tended to decline each day for the first six days of the disease among the recipients of serum, whereas among those who were not treated with serum the incidence of positive blood cultures tended to increase each day. After the sixth day the incidence of bacteremia among the serum-treated patients fluctuated from day to day and tended to approach that found among the patients treated without serum. Considering the results of blood cultures taken on different days of the disease up to the sixth day, the mortality was consistently lower among the serum-treated patients, whether the cultures were positive or negative. After the seventh day, however, the mortality was lower only for the patients who showed positive blood cultures (table 12).

The influence of serum in helping to sterilize the blood stream was reflected by the frequency with which positive blood cultures were followed by negative cultures, even when the disease was still in progress (table 13), by the rarity with which positive blood cultures were observed after previous cultures had remained sterile (table 13) and by the somewhat lower percentage of positive cultures of heart blood post mortem for the specifically treated patients (table 14).

It is to be emphasized that the patients listed here as serum-treated include all the patients receiving the homologous type of antibody, irrespective of the amount or the time of its administration. The importance of the dose of antibody and of the day of the disease when it is given are considered in detail elsewhere.[7]

COMMENT

Space does not permit a detailed discussion of all the possible implications and deductions which may be drawn from the data here pre-

sented. It is hoped that these findings will help to answer a number of questions with regard to the significance of bacteremia, which can be answered only after a laborious analysis of a large amount of material in some such manner as was here attempted.

Much of the earlier literature purported to show that pneumonia is always accompanied with bacterial invasion and that the divergent results obtained by different observers may be ascribed to the technics used in making the blood cultures.[11] Such a view would necessarily divest of any prognostic significance the finding of pneumococci in the blood of patients with pneumonia. There is considerably better agreement in the more recent literature in this regard; and the differences in the incidence of bacteremia and the mortality, while the figures may possibly vary for different localities or for different years,[4] are probably more affected by the choice of cases or by the attempts to draw conclusions from a small number of cases. The more recent knowledge concerning the biology of the pneumococcus [3a] has tended to standardize methods sufficiently to exclude this as an important factor.

SUMMARY

A series of 1,586 consecutive cases of pneumococcic pneumonia due to pneumococci of types I to XXXII (Cooper) in which blood cultures were made during life and/or at autopsy was analyzed with respect to the incidence of bacteremia and the mortality. The relation of the finding of positive blood cultures to other factors which are known or supposed to influence the prognosis has been considered. The effect of bacteremia on the occurrence of postpneumonic complications and its influence on the duration of the disease also were noted. Cases in which treatment with specific serum was given have been compared with cases in which no serum was given with respect to most of these subjects. The data are presented largely in tables, and the deductions are summarized in the text under the appropriate headings.

11. Prochaska, A.: Bakteriologische Blutuntersuchungen bei Pneumonien, Centralbl. f. inn. Med. **21**:1145, 1900. Rosenow, E. C.: Studies in Pneumonia and Pneumococcus Infections, J. Infect. Dis. **1**:280, 1904. Kinsey, F. C.: Examination of the Blood of Pneumonia Patients for Pneumococci, J. A. M. A. **42**: 758 (March 19) 1904.

THE BLOOD NITRITE

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Knowledge of the physiology of the nitrites is much confused. This may be attributed in part to the previous lack of a test sufficiently sensitive yet specific for detecting the presence of or measuring extremely minute quantities of nitrite. Very minute doses of nitrite have profound physiologic effects. Although an immense amount of investigation has yielded many significant observations, many enigmas still remain unsolved in this complex field.

Glyceryl trinitrate was discovered by Sobrero [1] in 1847, and the first physiologic studies on nitrites were initiated. Since then the accumulated literature has become enormous, and only a few significant papers can be reviewed here. Early analytic studies [2] revealed nitrite in many tissues but in some only after the tissues were ground. Liver reduces nitrate to nitrite.[3] The mechanism of this reduction is not definitely determined, but a vital process is presumably involved because this reduction is completely abolished by one five hundredth molar cyanide. Nitrite is found in human urine only in the presence of Bacillus coli infection of the urinary tract and never in normal urine. This fact has been applied as a diagnostic aid.[4]

The pharmacologic effects of nitrite are of two types: primarily, relaxation of smooth muscle and, secondarily, other physiologic responses, often due to changes in the circulation. The primary effect

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1. Sobrero, A.: Institut **15**:53 (Feb. 15) 1847.

2. Stepanow, A.: Arch. f. exper. Path. u. Pharmakol. **47**:411, 1902.

3. Bernheim, F., and Dixon, M.: Biochem. J. **22**:125, 1928.

4. Jaeggy, E., and Lanz, W.: Schweiz. med. Wchnschr. **65**:363, 1935. Machold, K.: Med. Klin. **30**:1097 (Aug. 17) 1934. Merkelbach, O.: Ztschr. f. klin. Med. **110**:427, 1929. Popper, L., and Weiss, S.: Wien. klin. Wchnschr. **41**:1081, 1915. Catel, W.: Jahrb. f. Kinderh. **140**:253, 1933.

is most conspicuous on the cells of the smooth muscles of the arterial and arteriolar walls. Secondary effects are observed in secretory organs which are affected by changes in the blood flow. For example, an increased rate of pancreatic secretion follows the administration of nitrite.[5] Nitrites cause a cessation of peristalsis of the intestines and relaxation of the stomach, with relief of pain due to hypertonicity.[6] This response has been employed in gastro-intestinal roentgenology to distinguish between spasm and organic constriction. Experiments with isolated strips of intestinal wall [6b] indicate that nitrite acts directly [7] on the smooth muscle of the intestines. The effect of nitrite on the smooth muscle of the uterus is less intense but of the same character.[8] Sodium nitrite has an effect similar to, though of lesser degree than, histamine in increasing gastric secretion.[9] This effect is due, no doubt, to the vasodilating action of the nitrite.

The administration of nitrite produces a fall in the level of the blood sugar in diabetic as well as in normal subjects, later followed by a rise in the concentration.[7] This has been explained as due to an increased diffusion of sugar out of the dilated capillaries; but sugar, which reduces nitrite, must itself be oxidized in the process. A fall in blood pressure stimulates the secretion of epinephrine and the consequent liberation of hepatic glycogen.[10] When amyl nitrite is administered to dogs the volume of the liver is reduced.[11] Conflicting reports confirm and deny that nitrites affect the rate of oxygen consumption.[12] Hyperpnea, which frequently follows the administration of nitrite, has been attributed to the direct action of the drug on the respiratory center.[13] There is also evidence [14] that nitrites affect the sensory endings in the carotid sinus and produce reflex hyperpnea.

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5. Barlow, O. W.: Am. J. Physiol. **81**:189 (June) 1927.

6. (*a*) Beams, A. J.: The Effect of Nitrites on Pain and on Motility of the Gastro-Intestinal Tract: I. Clinical Study, Arch. Int. Med. **49**:270 (Feb.) 1932. (*b*) Beams, A. J., and Barlow, O. W.: The Effect of Nitrites on the Motility of the Gastro-Intestinal Tract: II. Experimental Study, ibid. **49**:276 (Feb.) 1932.

7. Herzfeld, E., and Krüger, R.: Klin. Wchnschr. **8**:1174 (June 18) 1929.

8. Croft, C. R.: Lancet **2**:166, 1928.

9. Bergman, A.: Prensa méd. argent. **15**:1526 (May 20) 1929.

10. Tournade, A., and Chabrol: Compt. rend. Soc. de biol. **93**:934, 1925.

11. Edmunds, C. W.: J. Pharmacol. & Exper. Therap. **18**:155 (Sept.) 1921.

12. (*a*) Meyers, J.: Arch. d. mal. du cœur **19**:615, 1926. (*b*) Weiss, S., and Ellis, L. B.: Influence of Sodium Nitrite on the Cardiovascular System and on Renal Activity, Arch. Int. Med. **52**:105 (July) 1933.

13. Rosenblüth, E., and Wassermann, S.: Arch. f. exper. Path. u. Pharmakol. **144**:235, 1929.

14. Danielpolu, D., and Aslan, A.: J. de physiol. et de path. gén. **23**:572 and 606, 1925. Heymans, C.; Bouckaert, J. J., and Dautrebande, L.: Compt. rend. Soc. de biol. **106**:1279, 1931.

The use of glyceryl trinitrate in medicine followed Sobrero's discovery in 1847; Guthrie [15] introduced amyl nitrite in 1859, and sodium nitrite was employed clinically for the first time in 1883.[16] Arterial dilatation is so general that the soluble nitrites and alkyl nitrates cause a profound reduction of the arterial tension. Insoluble nitrate, as bismuth subnitrate, has been used as a vascular sedative in the treatment of hypertensive arterial disease with encouraging results.[17] Sollmann [18] has stated that vasodilatation due to nitrite probably is the result of a direct action on the smooth muscle of the walls of the arterioles, with no significant action on the capillaries and venules; but others [19] have suggested that the capillaries are affected. It has been stated also that the venules are dilated by nitrite.[20] With active hyperemia induced by arteriolar dilatation an increase in the capillary flow is inevitable. Densham [21] showed that lactate ion acts as an adjuvant to the vasodilator effect of sodium nitrite. The tone of the vessels also is affected directly by changes in the acid-base balance, but the effect of the lactate ion is specific and is not obtained with hydrochloric acid at a similar hydrogen ion concentration.

A reflex hypertension often follows vasodilatation produced by nitrite.[22] This vasoconstriction is explained as being due to a secondary stimulation of the vasomotor center resulting from the primary fall in blood pressure and consequent cerebral anemia.[23] It is said that the cardiac output is increased somewhat by the administration of nitrites,[24] but

15. Guthrie, F.: Quart. J. Chem. Soc. **11**:245, 1859.

16. Hay, M.: Practitioner **30**:180, 1883.

17. Stieglitz, E. J.: J. Pharmacol. & Exper. Therap. **32**:23 (Nov.) 1927; **34**:407 (Dec.) 1928; **46**:343 (Nov.) 1932; Bismuth Subnitrate in Treatment of Arterial Hypertension, J. A. M. A. **95**:842 (Sept. 20) 1930; Arterial Hypertension, New York, Paul B. Hoeber, Inc., 1930; Abnormal Arterial Tension, New York, National Medical Book Co., 1935. Bruen, C.: J. Lab. & Clin. Med. **18**:138 (Nov.) 1932.

18. Sollmann, T.: Manual of Pharmacology, ed. 2, Philadelphia, W. B. Saunders Company, 1926, p. 494.

19. Deusch, G., and Liepelt, A.: Deutsches Arch. f. klin. Med. **160**:207, 1928. Leake, C. D.; Kammer, A. G., and Hitz, J. B.: J. Pharmacol. & Exper. Therap. **35**:143 (Feb.) 1929. Schulman, E., and Marek, J.: Compt. rend. Soc. de biol. **95**:1474, 1926. Carrier, E. B.: Am. J. Physiol. **61**:528, 1922.

20. Payan, L., and Giraud, E.: Compt. rend. Soc. de biol. **93**:351, 1925.

21. Densham, B.: J. Physiol. **63**:175, 1927.

22. Gaucher, M.; Lequime, J., and Van Bogaert, A.: Compt. rend. Soc. de biol. **114**:1056, 1933. Dautrebande, L.: Arch. internat. de pharmacodyn. et de thérap. **43**:247, 1932. Burgess, A. M.: Ann. Int. Med. **5**:441, 1931.

23. Pilsher, J. D., and Sollmann, T.: J. Pharmacol. & Exper. Therap. **6**:323, 1915.

24. Gaisbock, F., and Jarisch, A.: Wien. klin. Wchnschr. **40**:1540, 1927.

this is denied by Weiss and Ellis.[12b] In this connection it should be mentioned that Weiss and Ellis observed no improvement in renal secretion following the administration of sodium nitrite, in contradistinction to the finding by Mason [25] that the rate of excretion of water by the kidneys is definitely increased secondary to an increased blood flow produced by sodium nitrite, provided the blood pressure is not too markedly lowered.[26] It must be emphasized that changes in the renal circulation are dependent not only on the systemic arterial tension but especially on the state of constriction or dilatation of the renal vessels. Nitrates have definite diuretic properties.[27]

A wholly unsolved problem is the question of why the doses required to produce a certain degree of vasodilatation should vary so markedly with the different nitrites and alkyl nitrates. One milligram of glyceryl trinitrate produces the same fall in arterial tension as does 40 mg. of erythrol tetranitrate, 50 mg. of sodium nitrite or 100 mg. of amyl nitrite. These wide differences cannot be accounted for on the basis of the amount of nitrite ion available. It has been assumed, but never proved, that ease of diffusion and cellular penetration are the chief variables. The alkyl nitrates act by liberation of the nitrite ion through hydrolysis.[28]

Tolerance to nitrites is acquired rather easily. Crandall and his collaborators [29] demonstrated an acquired tolerance and cross-tolerance between nitrous and nitric acid esters in man. The criterion of tolerance in their experiments was an increase in the dose of nitrite necessary to produce headache. There is a marked individual variation in the amount of nitrite required to cause headache, but before as well as after tolerance has developed the dose which will cause headache in any one subject is remarkably constant.[30] Tolerance to sodium nitrite is produced with difficulty and only after long administration of large doses.[31] Erythrol tetranitrate, glyceryl trinitrate, ethylene glycol dinitrate, methyl nitrate and amyl nitrite all produce a cross-tolerance.[29] This tolerance develops irrespective of the route of administration of the drug. The tolerance is to the chemical substance (presumably nitrite ion) and not to the physiologic response, for no cross-tolerance to other vaso-

25. Mason, E. C.: J. Lab. & Clin. Med. **9**:529, 1924.

26. Loeb, A.: Deutsches Arch. f. klin. Med. **94**:579, 1908.

27. Keith, N. M.; Whelan, M., and Bannick, E. G.: The Action and Excretion of Nitrates, Arch. Int. Med. **46**:797 (Nov.) 1930.

28. Hay, M.: Practitioner **30**:422, 1883. Crandall, L. A., Jr.: J. Pharmacol. & Exper. Therap. **48**:127, 1933.

29. Crandall, L. A., Jr.; Leake, C. D.; Loevenhart, A. S., and Muehlberger, C. W.: J. Pharmacol. & Exper. Therap. **41**:103, 1931.

30. Oltman, T.: Thesis, University of Wisconsin, 1927.

31. Myers, H. B., and Austin, V. T.: J. Pharmacol. & Exper. Therap. **36**:227, 1929.

dilators, such as histamine, is acquired. The tolerance, irrespective of the nitrite-forming drug used, lasts for about ten days after withdrawal. Tolerance is acquired most rapidly from those esters most slowly hydrolyzed by alkali.[32]

All these pharmacologic studies are of great interest, but they fail to illuminate whether or not nitrites exist as such in the body fluids in the normal state and are concerned with normal physiologic processes and whether the concentration of nitrite in the body fluids is actually increased after the administration of nitrites and/or nitrates. An extremely sensitive, accurate method of analysis is required for the detection and measurement of the nitrite content of the tissue fluids, for the concentration is extraordinarily minute. The development of a more highly sensitive test [33] made possible these studies on the nitrite content of the blood, spinal fluid, saliva, urine and sweat.

METHOD

By using alphanaphthylamine and the disodium salt of betanaphthylamine-6,8 disulfonic acid (known as amino-G acid)—an intense azo dye is produced in the presence of nitrite ions. The "amino-G" salt is substituted for the sulfanilic acid of the Ilosvay test.[34] The azo dye is intense violet in acid solution. Nitrite can be detected by this reaction in pure water in dilutions of 1:250,000,000 in the unknown. The test is applicable to quantitative colorimetric determinations because of the relative permanence of the color produced.

Nitrites are especially unstable and active in an acid solution; the liberated nitrous acid is readily destroyed, either by oxidation or by reduction. Certain bacteria destroy nitrite readily. It combines with hemoglobin to form methemoglobin. The nitrite content diminishes rapidly in sterile blood on standing in vitro. It is of the utmost importance, therefore, to avoid any destruction of nitrite in the unknown solution prior to the application of the test. When the method is applied to blood the specimen must be preserved so that the nitrite content is unaltered for several hours. It was found that the detectable content of nitrite could be preserved for at least twenty-four hours by mixing the blood with zinc sulfate [33] immediately on withdrawal. A modified Somogyi [34] technic for the precipitation of proteins was used throughout these experiments. Details of method and precautions to be observed with the use of this technic were described at length in an earlier paper.[33]

The test offers the following advantages over previously reported methods: (*a*) greater sensitivity, (*b*) uniformity of color for at least eighteen hours, (*c*) relatively stable reagents, which keep well, and (*d*) rapid maximum development of color.

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32. Herman, R. F.; Leake, C. D.; Loevenhart, A. S., and Muehlberger, C. W.: Proc. Am. Soc. Pharmacol. & Exper. Therap. **27**:259, 1926. Oltman, T. V., and Crandall, L. A., Jr.: J. Pharmacol. & Exper. Therap. **41**:121, 1931.

33. Stieglitz, E. J., and Palmer, A. E.: J. Pharmacol. & Exper. Therap. **51**: 398, 1934.

34. Ilosvay: Bull. Chem. **2**:347, 1889. Griess, P.: Ber. d. deutsche chem. Gesellsch. **12**:423, 1879. Somogyi, M.: J. Biol. Chem. **86**:655, 1930.

RESULTS

The test was applied to many samples of blood of normal human beings (table 1), and it was found that nitrites exist in normal blood with an average measured concentration of 0.8 microgram per hundred cubic centimeters. At times a sample from a subject who ordinarily showed some nitrite in the blood was found to give a negative result. In two subjects for whom frequent readings were obtained over a period of two and one-half years, it was noted that negative results were obtained during the warm months of the year. In another patient it was observed that the readings were negative during attacks of gout.

TABLE 1.—*Nitrite Content of Normal Blood*

Age	Sex	Arterial Tension	Nitrite Micrograms per 100 Cc.
23	M	118/ 80	0.8
72	F	190/ 20	0.7
52	F	170/110	0.6
23	M	115/ 70	1.1
25	M	125/ 85	1.5
25	M	125/ 80	1.2
27	M	125/ 80	0.5
22	M	125/ 75	1.1
22	M	122/ 75	0.9
20	M	120/ 80	0.6
20	M	122/ 75	1.6
38	M	140/ 90	0.6
21	M	124/ 80	1.5
23	F	124/ 85	1.0
24	F	120/ 82	0.8
24	F	120/ 85	0.7
24	F	120/ 80	0.0
34	M	120/ 85	1.1
34	M	118/ 85	1.3
34	M	115/ 85	0.0
35	M	110/ 80	0.5
35	M	114/ 85	0.0
23	M	118/ 75	1.3
23	M	120/ 70	0.8
23	M	135/ 82	0.6
50	M	140/ 85	0.0
Average			0.8

Normal urine contains no nitrite. Known amounts of a standard solution of nitrite were added to acid urine and to alkaline urine. The amount of nitrite which could be measured, as compared with the standard, was decreased in both, with a slightly greater decrease in the alkaline urine. It was observed also that pure solutions of phosphates decreased the amount of detectable nitrite.

The test was applied to saliva collected by means of a special tube fitting over Stensen's duct and was found to yield positive results of a magnitude comparable to that of the blood. Saliva not thus carefully collected is contaminated with bacteria and food particles, and the readings are far higher.

Spinal fluid was obtained from six patients and all six fluids gave negative results.

Tests were carried out on sweat also. Four healthy men were studied. They stayed in a hot room (140 or 150 F.) for four periods of ten minutes each, with one half to one hour of rest between periods. The skin was carefully cleansed with soap and water and then with alcohol and ether. The sweat was allowed to flow directly into a test tube containing the "amino G" acid reagent, and the alphanaphthlamine was added later. Each subject had imbibed a total of 1,500 cc. of a mixture of orange juice, lemon juice and water. Four successive samples of sweat were collected from each subject (table 2). It will be noted that in the first two subjects there was a progressive decrease in the concentration, whereas in the last two the concentration remained practically constant, at levels paralleling those found in blood.

The test was applied to the blood and spinal fluid of dogs, with consistently negative results. In rabbits both the blood and the urine showed negative results, although the blood gave positive readings after the administration of nitrite.

TABLE 2.—*Nitrite Ion in Sweat*

Subject	Nitrite Ion, Micrograms per 100 Cc.			
	Period 1	Period 2	Period 3	Period 4
A	1.5	0.8	0.0	0.0
B	2.0	1.0	0.0	0.0
C	1.5	1.5	1.3	1.5
D	1.6	1.5	1.5	1.4

Thus it is demonstrated that nitrite ion exists in minute but constant amounts in normal human blood, saliva and sweat but that we have been unable to find it in normal spinal fluid and urine. Analyses of the blood, spinal fluid and urine of dogs and rabbits gave negative results for nitrite. The probable sources of the normal blood nitrite are many.

Various foods contain nitrites and/or nitrates. Meats are occasionally preserved with nitrites or nitrates,[35] and many legumes and green vegetables contain nitrates. Persons working in glyceryl trinitrate plants or with other nitrates or nitrites absorb enough so that tolerance for the drug develops.[36] Certain bacteria, notably Bacillus coli and Bacillus lactis-aerogenes of the normal intestinal flora, reduce nitrate to nitrite; they do so with greater ease in a semisolid than in a solid

35. Kolbe, F.: Ztschr. f. Fleisch- u. Milchhyg. **39**:173, 1929. Mazé, P., and Mazé, P. J.: Compt. rend. Soc. de biol. **115**:15 and 16, 1934.

36. Laws, G. C.: The Effects of Nitroglycerin upon Those Who Manufacture It, J. A. M. A. **31**:793 (Oct. 1) 1898. Letts and Rea: J. Am. Chem. Soc. **105**: 1157, 1914. Ebright, G. E.: The Effects of Nitroglycerin on Those Engaged in Its Manufacture, J. A. M. A. **62**:201 (Jan. 17) 1914.

medium and least readily in a liquid medium.[37] Experiments were carried out with a strain of B. coli isolated from the intestinal tract of a human being to determine the ability of this organism to reduce sodium nitrate and bismuth subnitrate. It was found that these bacteria reduced these substances and produced relatively large amounts of nitrite. The bacteria also reduce nitrite, and, therefore, much of the nitrite formed is thus simultaneously destroyed.[38] Such reduction of nitrate to nitrite certainly occurs in the bowel when nitrate reaches the colon, with its myriads of B. coli.

Reduction of tissue nitrates is another source of nitrite. Nitrates exist in many tissues of the body.[2] Whelen [39] determined the amounts of nitrate and nitrite together as averaging about 1 mg. of nitrate nitrogen per gram of tissue, which reveals an ample tissue reserve of nitrate as a potential source of nitrite. Iron in the presence of dextrose is a factor in the reduction of tissue nitrate to nitrite.[40] Tests of nitrosodiethylamine for nitrite give a strong positive result. Thus, other organic substances may be considered as potential sources of nitrite. There are reducing substances in the blood itself which probably act on any nitrate absorbed. No appreciable variation in the nitrite in human blood was detected paralleling dietary variation with fasting or with a high intake of meat or vegetables.

Nitrite-forming or nitrite-containing drugs increase the nitrite content of the blood. Following the administration of sodium nitrite it was possible to demonstrate a rise in the concentration of nitrite in the blood. One subject, with an initial arterial tension of 122 systolic and 75 diastolic and a blood nitrite content of 0.86 microgram per hundred cubic centimeters, was given 0.12 Gm. of sodium nitrite. In one-half hour the arterial tension had dropped to 102 systolic and 65 diastolic, and the nitrite content had increased to 4.5 micrograms per hundred cubic centimeters. In one hour the arterial tension was 114 systolic and 70 diastolic, and the nitrite level was slightly reduced to 3.7 micrograms per hundred cubic centimeters. In another subject administration of sodium nitrite (0.18 Gm.) caused a rise in the blood nitrite which measured from 1 to 3.1 micrograms per hundred cubic centimeters in twenty minutes. Experiments in which bismuth subnitrate was administered orally substantiate the hypothesis that ingested nitrate is reduced to nitrite in the lower portion of the intestinal tract by nitrate-reducing

37. Zobell, C. E.: J. Bact. **24**:273, 1932.

38. (*a*) Buswell, A. M.: Chemistry of Water and Sewage Treatment, New York, The Chemical Catalog Company, Inc., 1928, p. 247. (*b*) Stieglitz, E. J., and Palmer, A. E.: J. Pharmacol. & Exper. Therap. **56**:216 (Feb.) 1936.

39. Whelen, M.: J. Biol. Chem. **86**:189, 1930; J. Lab. & Clin. Med. **20**:755, 1935.

40. Baudisch, O.: J. Biol. Chem. **48**:489, 1921.

bacteria and that the nitrite thus produced is absorbed into the blood stream as such, for the measurable nitrite content of the blood increases correspondingly.[38b]

The normal detectable nitrite content of the blood, however, is remarkably constant. It is highly probable that there is a continuous formation and simultaneous destruction of nitrite in the blood. Nitrite is rapidly destroyed by blood in vitro, and this destruction is due to a number of substances. Protein decreases the amount of nitrite which can be measured; the greater the amount of protein present, the greater the decrease in measurable nitrite. Nitrite and hemoglobin form stable methemoglobin. Precipitation of proteins from egg albumin and solution of nitrite reduces the detectable nitrite in the filtrate to but a fraction of the amount of nitrite originally added. Dialysis of serum or solutions of egg albumin were carried out against pure water, using a pyroxylin membrane, and after dialysis equal amounts of nitrite were added to the fractions. The portion remaining in the sac (undialyzable fraction) showed greater activity in destroying nitrite than did the dialysate. Nitrite is very active chemically and reacts readily with the amine groups of protein.

Dextrose also destroys nitrite. This is demonstrated readily in the test tube. In connection with the effect of sugar on the nitrite readings, the nitrite levels of normal and diabetic subjects were compared. In four diabetic patients, two of whom were receiving insulin and two of whom were not, with concentrations of sugar ranging from 183 to 330 mg. per hundred cubic centimeters of blood, no nitrite could be detected in the blood. In one subject with suspected diabetes, with a sugar content of 118 mg. per hundred cubic centimeters of blood, without insulin, a positive reading of 0.4 microgram per hundred cubic centimeters of blood was obtained. Further work along this line must be carried out to make these results convincing; these observations are merely suggestive. The administration of nitrates leads to a fall in the level of the blood sugar.[7]

It has long been suspected that normal blood contains thiocyanate ion, which has a powerful destructive action on nitrite. Both thiocyanates and nitrites are vasodilators. In synthetic protein and thiocyanate solutions it was shown that enough thiocyanate remains in the filtrate after protein precipitation to destroy added nitrite. Silver thiocyanate is highly insoluble. Modification of the technic by adding silver acetate immediately to freshly drawn blood gave slightly higher readings than those found in the same blood analyzed in the usual manner. Since minute amounts of thiocyanate will destroy relatively large amounts of nitrite (1 microgram of thiocyanate destroys 10 micrograms of nitrite); and since nitrite is actually found in the blood, this observa-

tion indicates that thiocyanate may exist in normal blood, but only in extremely minute amounts.

Comparison of control solutions and solutions containing urea in concentrations equivalent to those in the blood showed no difference in the measurable content of nitrite. Uric acid likewise had no effect. Even the addition of amounts of uric acid such as are observed in the blood in gout showed no diminution of the nitrite content. This is interesting in the light of our not being able to obtain nitrite readings for the blood of one subject with gout during periods of hyperuricacidemia. Epinephrine, however, actively destroys nitrite.

Pure amino-acetic acid, cystine, tyrosine, alanine and leucine were added to standard solutions of nitrite. No appreciable decrease in the concentration of nitrite was observed. Alcohol in vitro reduces nitrite. The nitrite content of the blood was found to fall from 0.8 microgram per hundred cubic centimeters to negative findings after several alcoholic drinks.

CONCLUSIONS

With a new sensitive specific test for nitrites studies have been made to determine whether nitrite ion exists in certain normal biologic fluids and what the factors are which control the amounts found in the blood.

It has been shown that nitrite is present in small amounts in the blood, saliva and sweat but not in the spinal fluid or normal urine of man. The concentration of detectable nitrite ion in normal human blood is of the order of magnitude of 1 : 100,000,000 during the cool months, and frequently none is found in hot weather. The concentration of nitrite in the blood has been shown to increase after the administration of sodium nitrite or bismuth subnitrate.

It has been shown that proteins, sugar, alcohol, phosphates, epinephrine and thiocyanate decrease the amount of measurable nitrite and that blood on standing even a few minutes shows a rapidly decreasing amount of nitrite available for the test. Nitrites are unstable chemically and are readily destroyed either by oxidation or by reduction. Certain bacteria destroy nitrite readily. Because of the rapid disappearance of nitrite from blood and the relative constancy of the level found in freshly drawn blood, one may tentatively assume that there is a constant production and destruction of the nitrite going on in the body. It also seems more than likely that only a fraction of the nitrite which may be in the blood stream is measured. The fact that the normal readings are relatively constant seems to indicate that perhaps an aliquot portion is obtained.

Amino acids, urea and uric acid do not seem to have any influence on the nitrite level of the blood.

The source of the nitrite of the blood may be from administration by any route, absorption of nitrites from the bacterial reduction of food or drug nitrates in the lower portion of the bowel or absorption of nitrates and a subsequent reduction in the blood stream itself or a reduction of nitrates in the tissues. Any of these sources may be foci of a more or less constant production of nitrite, probably in far greater amounts than those shown in the course of these experiments, because of the many factors constantly destroying the nitrite. Nitrite, as nitrous acid, has profound effects in very small amounts on a great many functions of the body directly by its action of relaxing smooth muscle, especially arteriolar muscle, and indirectly by its effects on the blood flow in secretory organs.

The exact physiologic significance of the blood nitrite is uncertain, but it may be that normally it aids in maintaining those functions which are stimulated by the administration of therapeutic doses. Clinical application of nitrite analysis of the blood may reveal some correlation between a disturbed nitrite metabolism and abnormalities of the arterial tension. With a new and far more sensitive test for minute quantities of nitrite, quantitative pharmacologic studies are now made possible.

ABSTRACT OF DISCUSSION

Dr. Anton J. Carlson, Chicago: My interest in this work arose from the fact that one is dealing here with a new normal constituent of the internal environment. The nitrites appear to be present in an amount similar to that of some of the vitamins and some of the hormones that have a profound effect in the body economy. The work has been difficult so far. I admire Dr. Stieglitz' persistence in contributing to fundamental medical science while making his living in medical practice. I think it is the result partly of heredity and partly of environment. He has opened up a new field here. I do not know whether it is going to be a blind alley or of profound significance, but nitrates given by mouth can apparently be converted to nitrites in sufficient amounts to produce significant physiologic effects.

Dr. Edward J. Stieglitz, Chicago: This work has been discouraging at times. Nitrite is so easily destroyed, and it is so easy for things to become contaminated. Ordinary tap water as it comes from Lake Michigan contains from a hundred to a thousand times as much nitrite as is found in the blood. Glassware left in the laboratory is contaminated with the fumes of nitrous acid. Glassware must be washed with cleaning solution and rinsed with fresh distilled water within half an hour of its use. The corollary of hormones going around to the frontal lobes did not occur to me, but the work has seemed like the music which goes around and around; very little comes out. Just one little toot has been heard so far, but I am convinced that there is a lot more music in this horn.

EFFECT OF SUNLIGHT ON THE CLINICAL MANIFESTATIONS OF PELLAGRA

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The influence of the sun's rays on the lesions of pellagra has been a subject of debate for nearly two hundred years. Casal [1] noted a seasonal variation in the incidence of pellagra, with a peak which corresponded to the spring equinox. In Italy one of the common names applied to this disease by the peasants is *mal del sole* (disease of the sun), while certain Italian physicians have described the lesions as due to "sunstroke of the skin." [2] Many modern clinicians [3] have stated

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From the Department of Medicine, Duke University School of Medicine, and Duke Hospital.

The various extracts of liver used and a grant of money for the free hospitalization of the patients presented in this study were supplied by Eli Lilly & Co., Indianapolis.

1. Casal, G., cited by Major, R. H.: Classic Descriptions of Disease, Springfield, Ill., Charles C. Thomas, Publisher, 1932, pp. 575-579.

2. Sambon, L. A.: Remarks on Geographical Distribution and Etiology of Pellagra, Brit. M. J. **2**:1272, 1905.

3. (*a*) Sambon.[2] (*b*) Bouchard, C.: Expériences relatives à la production de l'erythème solaire et plus particulièrement de l'erythème pellagreux, Compt. rend. Soc. de biol. **29**:253, 1877. (*c*) Thayer, W. S.: Note on Pellagra in Maryland, Bull. Johns Hopkins Hosp. **20**:193, 1909. (*d*) Wood, E. J.: The Appearance of Pellagra in the United States, J. A. M. A. **53**:274 (July 24) 1909. (*e*) Randolph, J. H.: Pellagra and Pellagrins, Arch. Int. Med. **2**:553 (Jan.) 1909. (*f*) Kimbrough, J. A.: Treatment of Pellagra, South. M. J. **5**:99 (Feb.) 1912. (*g*) Menage, H. E.: A Cursory Study of Skin Manifestations of Pellagra with Reference to Its Special Importance from a Diagnostic Standpoint, ibid. **5**:88 (Feb.) 1912. (*h*) Niles, G. M.: The Treatment of Pellagra, J. A. M. A. **62**:285 (Jan. 24) 1914. (*i*) Weston, W.: Pellagra in Early Childhood, Am. J. Dis. Child. **7**:124 (Feb.) 1914. (*j*) Voegtlin, C.: The Treatment of Pellagra, J. A. M. A. **63**:1094 (Sept. 26) 1914. (*k*) Wilson, W. T.: Management of Pellagra, ibid. **65**:1943 (Nov. 27) 1915. (*l*) Swanson, C.: The Differential Diagnosis of the Skin Manifestation in Pellagra from Other Dermatoses, South. M. J. **9**:117 (Feb.) 1916. (*m*) Bigland, A. D.: The Pellagra Outbreak in Egypt: I. Pellagra Among Ottoman Prisoners of War, Lancet **1**:947, 1920. (*n*) Klauder, J. V., and Winkelman, N. W.: Pellagra Among Chronic Alcoholic Addicts,

(Footnote continued on next page)

their conviction that there is a close relationship between exposure to sunlight and the development of cutaneous lesions in a pellagrin. Various observers [4] have produced typical cutaneous lesions in pellagrins by exposing normal or recently healed areas of skin to direct sunlight. Gherardini [3w] demonstrated the effect of the sun by systematically uncovering various parts of the body of each patient. Bouchard,[3b] Hameau [3x] and Ormsby [4a] had their subjects wear fenestrated gloves; Wilson [3k] had them expose both arms, and we [3y] have produced typical lesions in subjects by having each expose one arm to direct sunlight. Randolph [3e] prescribed a sun bath for a Negro with pellagra. After a short period of exposure the patient complained of intense burning of the skin, and he was promptly removed to a dark room; nevertheless, severe dermatitis appeared and was followed by stomatitis, diarrhea and dementia. The patient finally recovered.

The presence of lesions on unexposed portions of the body, such as the elbows, knees, sacrum, scrotum and perineum, has led certain students [5] of pellagra to deny the influence of sunlight in the production of the dermatitis. Tucker,[5c] Enright [5d] and Goldberger and Sebrell [5f]

J. A. M. A. **90**:364 (Feb. 4) 1928. (*o*) Manthos, C.: Pellagre d'abord atténuée (pellagroïde), démonstration de la photosensibilisation, Bull. Soc. franç. de dermat. et syph. **39**:1380, 1932. (*p*) Gougerot; Burnier, R., and Meyer, J.: Pellagre parisienne, ibid. **38**:847 (June) 1931. (*q*) Cluver, E. N.: Pellagra Among the Maize Eating Nations of the Union of South Africa, Brit. M. J. **2**:751, 1929. (*r*) Bass, C. C.: Pellagra, M. Clin. North America **12**:1181 (March) 1929. (*s*) Hartsock, F. M.: Radiant Energy: Sprue and Pellagra, Mil. Surgeon **66**: 523 (April) 1930. (*t*) Smith, J. H.: The Influence of Solar Rays on Metabolism, Arch. Int. Med. **48**:907 (Nov., pt. 2) 1931. (*u*) Corkill, N. L.: Pellagra in Sudanese Millet Eaters, Lancet **1**:1387, 1934. (*v*) Kirby, W. L.: The Eruption of Pellagra, South. Med. & Surg. **97**:11 (Jan.) 1935. (*w*) Gherardini, cited by Sambon.[2] (*x*) Hameau, cited by Sambon.[2] (*y*) Ruffin, J. M., and Smith, D. T.: The Treatment of Pellagra with Certain Preparations of Liver, Am. J. M. Sc. **187**:512 (April) 1934. (*z*) Neusser, E.: Die Pellagra in Oesterreich und Rumänien, Vienna, Alfred Hölder, 1887.

4. (*a*) Ormsby, O. S.: Pellagra, J. Cutan. Dis. **30**:589, 1912. (*b*) Bouchard.[3b] Randolph.[3e] Wilson.[3k] Gougerot, Burnier and Meyer.[3p] Gherardini.[3w] Hameau.[3x] Ruffin and Smith.[3y]

5. (*a*) Searcy, G. H.: An Epidemic of Acute Pellagra, J. A. M. A. **49**:37 (July 6) 1907. (*b*) Warnock, J.: Pellagra in Egypt, ibid. **53**:1664 (Nov 13) 1909. (*c*) Tucker, B. R.: Pellagra, with the Analytical Study of Fifty-Five Non-Institutional or Sporadic Cases, ibid. **56**:246 (Jan. 28) 1911. (*d*) Enright, J. I.: Pellagra Outbreak in Egypt, Lancet **1**:998, 1920. (*e*) Crutchfield, E. D.: Pellagra with Special Reference to the Skin and Mucous Membrane, Arch. Dermat. & Syph. **17**:650 (May) 1928. (*f*) Goldberger, J.: Pellagra, revised by W. H. Sebrell, in Tice, F.: Practice of Medicine, Hagerstown, Md., W. F. Prior Company, Inc., 1931, vol. 9, pp. 205-231. (*g*) Long, J. D.: Pellagra, J. A. M. A. **55**:734 (Aug. 27) 1910. (*h*) Spies, T. D.: Relationship of Pellagrous Dermatitis to Sunlight, Arch. Int. Med. **56**:920 (Nov.) 1935.

recognized that an existing lesion can be accentuated by exposure to sunlight, while Spies [5h] stated that the cutaneous lesions heal equally well regardless of whether the patient is kept in the dark or exposed to direct sunlight. Bigland [3m] and Spies [5h] failed to produce lesions in pellagrins by exposing them to sunlight, and Gougerot, Burnier and Meyer,[3p] Crutchfield,[5e] Bassi [6] and Spies [5h] reported that they noted no unfavorable response to ultraviolet radiation.

In an effort to determine which of these conflicting views is correct, an investigation of the subject was begun at Duke Hospital in 1931. It soon became apparent in taking the histories that a majority of patients, when specifically questioned, recalled a prolonged exposure to sunlight shortly before the appearance of the cutaneous lesions. It was noted also that the occurrence of dermatitis was almost invariably followed by constitutional symptoms of varying intensity. In only

TABLE 1.—*Relationship of Constitutional Symptoms to the Appearance of Dermatitis in One Hundred and Thirty Cases of Pellagra*

Symptom	Occurrence Before Dermatitis		Occurrence Coincident with or After Dermatitis	
	No. of Cases	Percentage	No. of Cases	Percentage
Sore mouth and tongue	17	13	93	72
Diarrhea	12	9	66	51
Abdominal pain	5	4	16	12
Nausea and vomiting	6	5	25	19
Anorexia	6	5	37	29
Nervousness	6	5	32	25
Mental disturbance	0	0	21	16
Paresthesia	2	2	17	13
Ataxia	0	0	8	6

seventeen of one hundred and thirty consecutive patients with pellagra were the mouth and tongue sore before the appearance of dermatitis. In ninety-three these symptoms were noted coincident with or appeared after the development of cutaneous changes. Diarrhea was present in twelve cases before and in sixty-six after the appearance of cutaneous lesions. The relationship of the most important symptoms to the appearance of dermatitis is shown in table 1.

These findings suggested that the severe constitutional symptoms of pellagra are related in some way to the action of sunlight on the exposed surfaces of the body. Thirty-five patients with clinical pellagra were hospitalized with the object of determining accurately the order of appearance of symptoms after controlled exposure of a portion of the body to the direct rays of the sun. The ordinary hospital diet, with an abundance of fresh red meat, milk, fruit and vegetables, is exactly the diet which Goldberger recommended for the cure of the disease.

6. Bassi, U.: Porphyrin in Pathogenesis of Pellagra, Clin. med. ital. **65**:241, 1934.

TABLE 2.—*Standard Basic Diet 1*

	Quantity, Gm.	Protein, Gm.	Fat, Gm.	Carbohydrate, Gm.	Minerals, Gm.			Vitamins					Calories
					Calcium	Phosphorus	Iron	A	B	C	D	G	
Corn meal	92	8.3	2.0	69.0	0.0110	0.1225	0.0006	..	±	..	..	±	.....
Cane syrup	105	....	....	89.2	.....	.....	.....	..	+	..	..	+	.....
Flour	111	12.5	1.2	83.4	0.0220	0.1030	0.0010	..	+	..	..	—	.....
Lard	81	....	81.0	....	.....	.....	.....	..	..	..	..	..	.....
Rice	25	2.0	0.1	19.6	0.0023	0.0240	0.0002	..	..	..	..	..	.....
Field peas	90	19.2	1.4	54.6	0.0756	0.0760	0.0052	+	++	..	..	+	.....
Hominy grits	51	4.3	0.3	40.6	0.0056	0.0734	0.0005	+	++	..	..	+	.....
Fat salt pork	60	1.1	51.3	0.0	0.0011	0.0115	0.0001	..	..	..	..	..	.....
													2,890.0
Cod liver oil, cc	90	....	90.0	....	.....	0.0117	.....	++++	..	..	++++	..	810.0
Tomato juice, cc	45	0.4	0.1	2.0	0.0070	.....	0.0002	++	++	++++	..	++	10.3
Ammonium citrate and iron	6	....	....	....	.....	.....	1.0200	..	..	..	..	..	.....
Calcium gluconate	6	....	....	....	0.5580	.....	.....	..	..	..	..	..	.....
Cheese	60	17.4	21.6	....	0.5586	0.4098	0.0007	++	++	..	..	..	264.0
Total		65.2	249.0	358.40	1.2392	0.8319	1.0285	++++	+++	++++	++++	+	3,974.3

Therefore, it was necessary to devise a basal diet which was adequate in protein, fat, carbohydrate, calories, minerals and all the vitamins except the pellagra-preventive factor of Goldberger. This diet was called standard basic diet 1 and is detailed in table 2. Although it is not entirely deficient in the pellagra-preventive factor, it produced black tongue when fed to dogs.[3y] The last ten patients in this series received standard basic diet 2, which was identical with diet 1 except that tomato juice was replaced by 90 mg. of cevitamic acid.

Patients with moderately severe pellagra were admitted to the hospital, fed the standard basic diet and protected from the direct rays of the sun. The cutaneous lesions usually improved under this regimen. In some cases the constitutional symptoms also subsided; in others they remained the same or became accentuated.

After the preliminary period of observation one hand and forearm and in some cases the corresponding foot and lower portion of the leg were exposed to the direct rays of the sun on from three to five consecutive days. The first exposure was usually for twenty or thirty minutes, and the time was increased by fifteen minutes each day up to a maximum of one hour. Members of the staff and patients with other diseases were used as controls, and in no instance was the exposure severe enough to produce a sunburn. Dermatitis appeared over the exposed areas in thirteen of the thirty-five cases (table 3). Two patients in whom no dermatitis was observed showed constitutional symptoms. No accentuation of the clinical manifestations of pellagra was noted in the remaining twenty patients.

The more important constitutional symptoms which appeared in the fifteen patients after exposure to the direct rays of the sun are shown in tables 4 and 5. The dermatitis which was observed in thirteen of these patients varied from erythema with vesiculation to heavily crusted indurated lesions and was indistinguishable from the cutaneous lesions of spontaneous pellagra (fig. 1).[7] In eleven instances the lesions were produced on a previously involved area. In eight patients, however, the dermatitis was produced on apparently normal skin which had previously been protected by clothing (table 3). The condition of the tongue became definitely worse in twelve of the fifteen patients (table 4). Diarrhea was precipitated in ten (table 5), nausea in seven, vomiting in six, anorexia in eight and dementia in four. In all the fifteen patients one or more severe constitutional symptoms developed after exposure to direct sunlight. Neither cutaneous lesions nor general symptoms occurred in any of the eleven patients who were reexposed to maximum doses of sunlight following adequate treatment (tables 3 to 5).

7. For other experimentally produced lesions see figures 1 to 3 in article by Ruffin and Smith.[3y]

TABLE 3.—*The Occurrence of Dermatitis Following Exposure to Direct Sunlight: Data for Thirty-Five Patients in the Hospital Subsisting on a Diet Deficient in the Pellagra-Preventing Factor*

Case	Age	Sex	On Admission	Before Exposure	After Exposure: Over Original Lesions	After Exposure: Over New Areas	Exposure After Treatment
1*	66	M	++	+	+	—	—
2*	31	F	+	+	+	—	—
3	9	F	+	+	+	—	—
4	32	F	+	0	0	—	—
5	50	M	+	0	0	—	—
6	21	F	+	0	0	—	—
7	27	F	+++	0	0	—	—
8	27	F	+	0	0	—	—
9*	20	F	+++	0	0	—	—
10	29	F	+	0	0	—	—
11	14	F	+	0	0	—	—
12	30	F	+	0	0	—	—
13	41	F	+	0	0	0	—
14	29	F	++	0	0	0	—
15	23	M	+	0	0	0	0
16	31	F	+	0	0	0	0
17	38	F	+	0	0	0	0
18	55	F	+	0	0	0	0
19	41	F	+	0	0	0	0
20*	45	F	++	0	0	0	0
21†	39	M	++	0	0	0	0
22†	39	F	++	++	++	0	0
Total: 22 cases							
23	25	F	++	++	++++	++++	0
24	35	M	+	0	++	++	0
25†	43	M	+	+	+++	+	0
26	8	M	++	0	+++	+++	0
27	54	M	+++	+	0	+++	0
28	34	F	+	+	++	—	0
29	54	M	++	+	++	—	Died
30†	42	M	++++	++	—	+	Died
31	76	M	+	0	+	++	Died
32	55	F	+	+	++	+++	0
33*	24	F	+++	+	++	—	0
34†	26	F	++++	+	++	0	0
35	42	F	++	±	+++	0	Died
Total: 13 cases							

* Negro patient.
†Alcoholic patient.

TABLE 4.—*The Occurrence of Sore Mouth and Tongue After Exposure to Direct Sunlight: Data for Fifteen Patients Subsisting on a Diet Deficient in Pellagra-Preventing Substance*

Case	On Admission	Before Exposure	After Exposure	Reexposure Treatment
21†	+++	0	++	0
22†	+	+	++	0
33*	+	+	++	0
23	+	+	++	0
32	+	+	++	0
31	+	+	++‡	Died
30†	+	+	++	Died
29	++	+	++	Died
28	0	0	0	0
27	++	+	+	0
26	+	0	++	0
25†	0	0	++§	0
24	+	0	0	0
34†	++	0	+++	0
35	+	+	+++	Died

* Negro patient.
† Alcoholic patient.
‡ Occurred ten days after exposure.
§ Scrotal lesions appeared for first time.

The following case history illustrates the severe reaction which may follow exposure of a pellagrin to sunlight and the harmlessness of such exposure after the deficiency has been corrected by adequate dietary treatment.

CASE 32.—N. C., a white woman aged 55, was admitted to the hospital on April 9, 1934, with a history of recurrent pellagra over a period of twenty years. At the time of entry mild dermatitis was present over the dorsum of each hand, the tongue was red and sore and the patient had little or no appetite and was somewhat constipated. She was given standard basic diet 1 (table 2).

On the second, third, fifth and sixth days after the patient's admission to the hospital the left arm and leg were exposed to direct sunshine for one hour daily.

TABLE 5.—*The Occurrence of Diarrhea After Exposure to Direct Sunlight: Data for Fifteen Patients Subsisting on a Diet Deficient in Pellagra-Preventing Substance*

Case	On Admission	Average No. of Stools Three Days Before Exposure	Average No. of Stools Three Days After Exposure	Reexposure After Treatment (Average No. of Stools Three Days Before Discharge)
21†	10	8	6	6
33*	3	2	4	4
23	1	1	9	1
32	1	1	5	2
31	0	1	1‡	Died
30†	2	4	13	Died
29	4	8	11	Died
28	0	0 (Enema)	3	1
27	0	1	1	2
26	0	0 (Enema)	1	1
25†	1	1	1	1
24	0	4	6	4
34†§	1	1	1	1
22†	5	3	9	1
35¶	5	7	10	Died
Average	2	3	5	2

* Negro patient.
† Alcoholic patient.
‡ Diarrhea occurred ten days later (average of five stools).
§ No diarrhea; on the fourth day of exposure severe nausea and vomiting were noted.
¶ Violent nausea and almost continuous vomiting occurred after the second day of exposure.

There was no involvement of the skin of the leg before the exposure. On the day of the last exposure the dermatitis over the left arm became much worse, and large blisters appeared over the foot. The control, exposed simultaneously, showed no sign of sunburn. By this time the rash on the right arm, which had been protected from the sun, had entirely disappeared. A few days later the tongue became fiery red, and severe diarrhea developed, twenty stools being passed on the fifth day after the last exposure. The patient's general condition became much worse. She was given preparations of liver, as shown in figure 2, but rapidly became worse. She became markedly depressed and eighteen days after the last exposure was disoriented, having definite auditory and visual hallucinations. At this time she was given the regular hospital diet and large doses of an aqueous solution of liver extract.[8] Within four or five days she was greatly improved,

8. This extract, known as Valentine's liver extract E 29, was furnished by the Valentine Meat Juice Company, Richmond, Va.

Fig. 1.—Photograph showing the lesions on the hands of a boy with pellagra. After he had been in the hospital for ten days on the diet detailed in table 2 the hands were exposed to direct sunlight daily for thirty, forty-five and sixty minute intervals. The lesions shown in the photograph developed subsequently to this exposure.

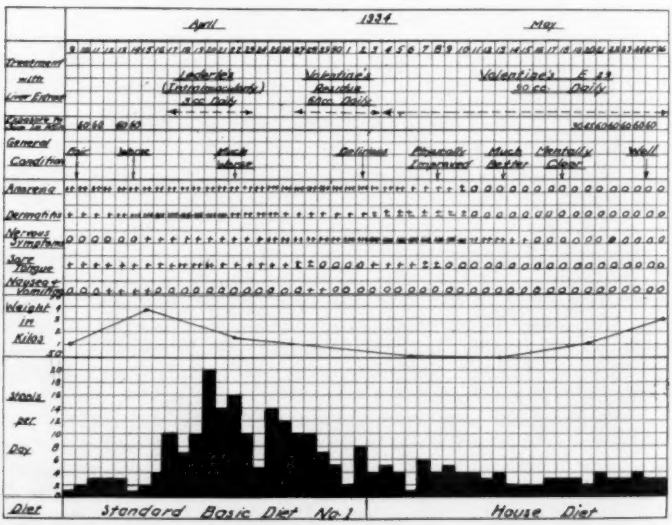

Fig. 2.—Chart showing the effect of exposure to sunlight on the clinical manifestations of the disease in case 1.

and after ten days she was mentally clear and felt entirely well. She was reexposed to direct sunshine daily for seven days, with no recurrence of symptoms, and was discharged on May 26, all evidences of pellagra having completely disappeared.

Niles [3h] and Bass [3r] have reported the development of dermatitis in pellagrins after exposure to heat from a stove. In one of our patients typical pellagra with severe constitutional symptoms followed exposure of the hands to a red hot stove. Another patient presented a similar picture after fighting a brush fire. These observations suggest that the acute manifestations of pellagra in a susceptible subject may be precipitated by exposure to the heat from certain radiant bodies. In the following case the typical cutaneous lesions and associated constitutional symptoms were produced by exposing the hands to the rays of an electric heater. After adequate dietary treatment the patient was reexposed to three times the original amount of heat, without the development of either cutaneous lesions or general symptoms.

CASE 36.—W. B. B., a white man aged 45, was admitted to the hospital on Nov. 26, 1935. For six months he had been drinking about 2½ gallons (9.5 liters) of whisky a week, and his diet had been grossly inadequate. He presented a typical picture of pellagra, with minimal cutaneous lesions over the dorsum of each hand, a beefy red tongue and marked anorexia but no diarrhea. Mild peripheral neuritis also was present. He was given a diet deficient in the pellagra-preventing factor.

On the day after his admission to the hospital and again on the following day the patient's left hand was exposed to the rays from an electric coil heater for fifteen minutes at a distance of 36 inches (92 cm.). Twenty hours after the first exposure distinct erythema was observed, and vesicles appeared on the morning following the second exposure. The hand of the intern who acted as a control showed no changes. On the day following the last exposure the patient's condition became alarming. The tongue was then fiery red, diarrhea, nausea and vomiting had developed and the patient refused to eat. He was given intravenous injections of dextrose and large amounts of an aqueous solution of liver extract by mouth. For several days there was considerable doubt whether he would survive. The stomatitis began to subside after the second day of this treatment, and by the fourth day the color of the tongue was normal. After five days of dietary treatment the patient's condition was definitely improved, and by the tenth day he was well except for persisting neuritis. One hand was then reexposed daily for five days to the electric coil heater for from fifteen to thirty minutes with no visible local or general effect. The patient was discharged as cured on December 17.

COMMENT

The clinical impression that sunlight is harmful to the pellagrin has been amply confirmed by observations on patients under controlled conditions in this hospital. Thirty-five pellagrins on a deficient diet (table 2) were exposed to the direct rays of the sun. In thirteen dermatitis developed, which was followed promptly by moderate or marked accentuation of the constitutional symptoms. Two other patients

showed similar constitutional reactions but no new cutaneous lesions. The first change usually noted was that the tongue became fiery red, followed by vomiting, diarrhea and, in some instances, dementia. The danger of exposing susceptible pellagrins to the sun's rays cannot be overemphasized, despite Spies'[5h] recently published conclusions that pellagrins recover equally well whether exposed to sunlight or kept in the dark.

The amount of exposure in each case was not excessive. This is shown by the failure of sunburn to develop in members of the staff and patients with other diseases who were exposed similarly and simultaneously. Furthermore, eleven of the fifteen patients who showed acute symptoms after exposure to sunshine were reexposed after the administration of an abundance of the pellagra-preventing factor, and in no instance did either dermatitis or constitutional manifestations recur. This demonstration of tolerance for direct sunlight is thought essential in determining whether the condition has actually been cured. The suggestion that sunburn may be confused with the dermatitis of pellagra is not in accord with the facts as we have observed them. Contrary to the opinion of Spies,[5h] experienced physicians in the South have no difficulty in making this distinction (Thayer,[3c] Menage,[3g] Swanson,[3l] Kirby[3v] and Wood and Taylor[9]).

The fact that twenty of the thirty-five patients showed nothing to suggest an accentuation of the dermatitis or constitutional symptoms after exposure to sunlight is difficult to explain. However, it was noted that without exception the patients who ate the basic diet with relish promptly recovered and showed only a healthy tan on exposure to sunshine.

In the group of twenty patients in whom no symptomatic relapse was observed, any attempt to test the efficacy of a curative substance would obviously have been futile. This serves to emphasize the importance of producing an accentuation of symptoms as a prerequisite to the evaluation of any curative substance. The failure to take this fact into consideration probably accounts for the voluminous literature on therapy and the numerous unrelated substances which have been reported as specific cures for pellagra.

The cutaneous lesions which were produced experimentally by graded exposures to direct sunlight were apparently identical with the spontaneous lesions, first described by Casal.[1] In addition to this typical dermatitis which appears on the exposed surfaces, patients with pellagra may present (1) seborrhea over the face and neck, (2) hyperkeratoses

9. Wood, E. J.: Pellagra, revised by F. R. Taylor, in Christian, H. A., and Mackenzie, J.: Oxford Medicine, New York, Oxford University Press, 1936, vol. 4, pt. 1, pp. 307-352.

over the bony prominences of the body and (3) lesions about the genitalia. The seborrhea occurs most commonly about the alae nasi but at times over the forehead, face and neck. The orifices of the sebaceous glands are plugged with a dry grayish yellow secretion. This type of lesion has been noted previously in pellagrins.[10] An analogous alteration in the sebaceous glands of the rat's tail has been produced by a diet deficient in vitamin G (B_2).[10c] There is no relation between the presence of seborrhea and the severity of the disease.

When a pellagrous patient is confined to bed, there is a tendency toward the development of bilateral symmetrical lesions over the bony prominences of the body, such as the elbows, knees, ankles and spinous processes. This lesion apparently is a hyperkeratosis, accompanied with more or less pigmentation. It differs from dermatitis, which occurs on

Fig. 3.—Photograph of hyperkeratosis of the elbow which developed while the patient was in the hospital and receiving an excellent diet. She showed no evidence of pellagra.

the exposed surfaces, by its slow development and by the absence of vesiculation. These lesions rarely, if ever, show crust formation, induration or secondary infection, and their development is not accompanied with an increase in constitutional symptoms. Bilateral symmetrical cutaneous lesions of this type have been frequently observed in both white and Negro patients who were receiving an adequate diet and who showed no evidence of pellagra (fig. 3). The cutaneous

10. (*a*) Castellani, A., and Chalmers, A. J.: A Manual of Tropical Medicine, ed. 3, London, Baillière, Tindall & Cox, 1919. (*b*) Biggam, A. G., and Ghalioungui, P.: Pellagra: Its Clinical Features and Pathology, Lancet **2**:1198, 1933. (*c*) Smith, S. G., and Sprunt, D. H.: Pathological Skin Changes in the Tail of the Albino Rat on a Diet Deficient in Vitamin G, J. Nutrition **10**:481 (Nov.) 1935. (*d*) Wood.[9] Bigland.[3m] Corkill.[3u] Bass.[3r]

lesions which Spies [5h] "produced" in pellagrins who were confined to the hospital and fed a deficient diet may have been of this type. He recorded no increase in constitutional symptoms accompanying the development of dermatitis. We agree with Bass [3r] in attributing this type of lesion to mechanical rubbing or pressure over the bony prominences of the body.

The male pellagrin may show lesions on the scrotum or perineum, although perineal lesions are more commonly seen in the female. Perineal lesions are red and macerated and invariably show secondary infection. The vaginal mucosa becomes inflamed in the same manner as the buccal and intestinal mucosa and pours out an irritating secretion. Perineal lesions in the male may be due to constant soiling with foul liquid stools and in the female to this factor supplemented by infected secretions from the vagina. Bass [3r] stated that the perineal lesions are caused by excoriating secretions and that the occasional lesions found under a pendulous breast are caused by excessive perspiration.

Our observations support the view of Goldberger [11] that pellagra is a deficiency disease. The basic deficiency symptoms are easy to recognize as due to "ill health" but difficult to classify. The clinical picture is one of gradually developing lassitude, anorexia, mental torpor, emotional instability, vague digestive symptoms, weakness and dizziness, sometimes with, but more generally without, marked loss of weight. The tongue frequently shows papillary atrophy, especially about the edges. In some cases there is roughness of the skin of the alae nasi and over the forehead and face, and hyperkeratosis may appear in the region of the bony prominences. Although pellagra may be suspected from these symptoms and treated as such, yet we agree with Wood [3d] and Bass [3r] that it is hazardous to make a diagnosis of pellagra in the absence of typical dermatitis or a credible history of such a dermatitis on the exposed surfaces of the body.

The reactions which occur after a patient in the deficient state becomes exposed to the sun are dramatic in onset, alarming in severity and not infrequently fatal. The usual story is that a subject who is subsisting on a deficient diet, with many of the vague symptoms already mentioned, though still able to work, exposes himself to the direct rays of the sun early in the spring or summer. Within a few hours a burning and itching sensation is noted over the exposed areas. This is followed in from twenty-four to thirty-six hours by a fiery red erythematous rash. Vesiculation appears, accompanied with induration, dry crusts

11. Goldberger, J.: Pellagra: Causation and a Method of Prevention, J. A. M. A. **66**:471 (Feb. 12) 1916.

or secondary infection. The bright red erythema changes to a dull bronze and finally to a deep brown. After the initial erythema the mouth and tongue become red and sore, and diarrhea appears. Dementia may develop without warning and is always an ominous sign.

It should be noted that in two patients with spontaneous pellagra and in one patient under controlled conditions radiant heat from sources other than the sun also produced the clinical picture of the disease.

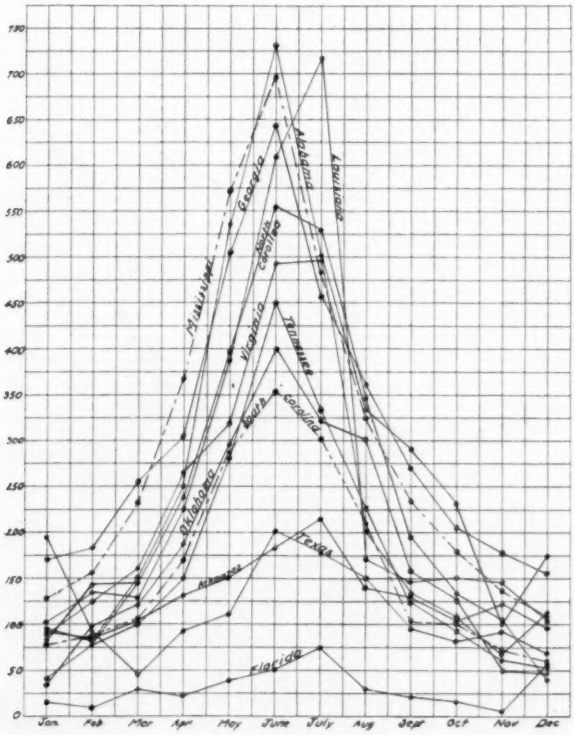

Fig. 4.—Chart showing the monthly incidence of pellagra in eleven Southern states over a period of five years (1930 to 1934). In the twelfth state (Arkansas) the monthly mortality is shown. To obtain the actual figures for Mississippi and South Carolina multiply by 10.

The seasonal incidence of pellagra (fig. 4) is apparently conditioned by two factors: the degree of dietary deficiency and the intensity of the solar radiation. In figure 5 the monthly variations in the intensity of sunlight are shown together with the monthly incidence of pellagra.

As Goldberger and his associates [12] have pointed out, there is a gradually accumulating deficiency of accessory food factors during the winter months. When these deficient patients become exposed to the increasing intensity of sunlight during the spring and early summer, the acute symptoms of pellagra appear. When vegetables and milk become more

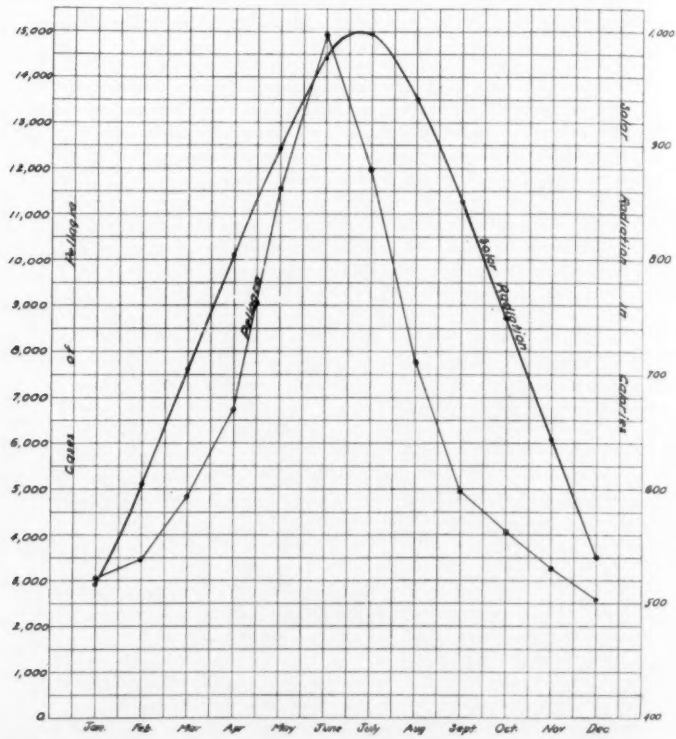

Fig. 5.—Chart illustrating the relationship of the intensity of the sun's rays to the incidence of pellagra. One curve shows the total monthly incidence of pellagra over a period of five years in the eleven states shown in figure 4. The curve for the total solar radiation is shown in calories per square centimeter per day on a surface at normal incidence at sea level on cloudless days for 33 degrees latitude and was calculated for us by Prof. Paul Gross, of the department of chemistry of Duke University, from the curves presented by C. G. Abbot in his book entitled "The Sun" (New York, D. Appleton-Century Company, Inc., 1929, p. 385, fig. 71).

12. Goldberger, J.; Wheeler, G. A.; Sydenstricker, E., and King, W. I.: A Study of Endemic Pellagra in Some Cotton-Mill Villages of South Carolina, United States Public Health Service, Hygienic Laboratory Bulletin 153, January, 1929.

plentiful during the summer, the deficiencies are corrected and the incidence of pellagra falls rapidly, although the sun remains hot during the month of July and August.

CONCLUSION

Exposure of a susceptible subject who has been subsisting on a deficient diet to the sun's rays precipitates the acute manifestations of pellagra.

Statistical data on the incidence of pellagra were supplied by Dr. J. C. Knox, of the North Carolina State Board of Health; Dr. D. G. Gill, of the Alabama State Board of Health; Dr. I. C. Riggins, of the Virginia State Board of Health; Dr. C. M. Pearce, of the Oklahoma State Board of Health; Dr. C. D. Reece, of the Texas State Board of Health; Dr. Butler Toombs, of the Georgia State Board of Health; Dr. C. L. Brown, of the Louisiana State Board of Health; Dr. F. A. Brink, of the Florida State Board of Health; Dr. R. N. Whitfield, of the Mississippi State Board of Health; Dr. W. C. Williams, of the Tennessee State Board of Health; Mrs. J. B. Collie, of the Arkansas State Board of Health, and Dr. M. B. Woodward, of the South Carolina Board of Health.

UVEOPAROTITIS

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The syndrome which is variously designated as uveoparotid fever, uveoparotitic paralysis, uveoparotitis and uveoparotid tuberculosis was brought to the attention of clinicians by Heerfordt [1] in 1909. Since then approximately eighty cases have been reported in the literature, of which not more than seven occurred in the United States. The earlier reports appeared largely in the Scandinavian and German journals of ophthalmology and apparently escaped the attention of the general medical practitioner. Credit is due to the English for reviving interest in the syndrome recently, especially through the medium of the review of Garland and Thomson [2a] published in 1933. Prior to that, and in the period from 1925 to 1931, only four cases had been reported in the United States (Cutler, Jackson, Hamburger and Schaffer [3] and Merrill and Oaks [4]). Subsequently, Levin,[5] Cogan [6] and Cohen and Rabinowitz [7] brought the number of cases reported in this country to seven, and additional reports came from Davies,[8] Rees,[9] Tait,[10] Garland and Thomson,[2] Tanner and McCurry,[11] and Savin [12] in England, Mohn [13] in Denmark and Haferkorn [14] in Germany.

From the Syphilis Division of the Medical Clinic, the Johns Hopkins Hospital.

1. Heerfordt, C. F.: Ueber eine Febris uveoparotidea subchronica, Arch. f. Ophth. **70**:254, 1909.

2. (*a*) Garland, H. G., and Thomson, J. G.: Uveoparotid Tuberculosis, Quart. J. Med. **2**:157, 1933. (*b*) Garland, H. G.: Uveoparotid Tuberculosis, Lancet **2**:743 and 846, 1934.

3. Hamburger, L. P., and Schaffer, A. L.: Uveoparotid Fever as a Manifestation of Mikulicz's Syndrome, Am. J. Dis. Child. **36**:434 (Sept.) 1928.

4. Merrill, H. G., and Oaks, L. W.: Uveoparotitis, Am. J. Ophth. **14**:15, 1931.

5. Levin, Paul M.: The Neurological Aspects of Uveo-Parotid Fever, J. Nerv. & Ment. Dis. **81**:176, 1935.

6. Cogan, David G.: Uveoparotid Fever, Am. J. Ophth. **18**:637, 1935.

7. Cohen, S. J., and Rabinowitz, M. A.: Uveoparotitis, J. A. M. A. **105**:496 (Aug. 17) 1935.

8. Davies, T. A. L.: Uveo-Parotitis Polyneuritica, Lancet **2**:746, 1934.

9. Rees, W. E.: Mikulicz' Syndrome in Association with Tuberculosis, Lancet **2**:749, 1934.

10. Tait, C. V. B.: Uveoparotitis, Lancet **2**:748, 1934.

11. Tanner, S. E., and McCurry, A. L.: Uveoparotid Tuberculosis, Brit. M. J. **2**:1041, 1934.

12. Savin, L. H.: An Analysis of the Signs and Symptoms of Sixty-Six Published Cases of the Uveoparotid Syndrome, Tr. Ophth. Soc. U. Kingdom **54**:549, 1934.

(Footnotes continued on next page)

The extensive reports of Garland and Thomson [2a] (1933) covering forty-seven cases in the literature and of Savin [12] (1934) analyzing sixty-six cases and the neurologic study of sixty-six cases by Levin [5] (1935) render superfluous any attempt to review the literature at this time. Accordingly, a summary of the commoner findings will suffice in this discussion.

The syndrome consists of inflammatory lesions of the uveal tract, enlargement of the parotid glands and a chronic low grade fever. Paralysis of cerebrospinal nerves, particularly the facial nerves, occurs frequently.

The majority of patients have been in the second and third decades of life, with a preponderance of females.

Prodromes, which may be present for a few days or for several weeks, include malaise, weakness, lassitude, drowsiness, nausea, vomiting, loss of weight, fever, puffiness of the eyelids, dryness of the mouth, dysphagia, cough, night sweats, pains in the chest and joints and paresthesias. Not infrequently early in the illness there is a patchy, nonelevated erythema, without itching, involving particularly the legs. The eruption is generally of short duration.

Parotitis is a constant finding. It is usually bilateral, only occasionally being unilateral. The gland is hard and not adherent to the skin, and while not painful, it may be tender to pressure. The swelling is usually of a few weeks' duration but may last for months. No suppuration occurs.

Chronic low grade fever is common but is not a constant finding. The temperature may range from the slightest elevation to from 101 to 103 F. in some instances. An afternoon elevation is common.

In addition to parotid enlargement, submaxillary and sublingual involvement is common, and lacrimal swelling occurs occasionally. Generalized lymphadenopathy and splenomegaly have been reported. The commonest finding is enlargement of the cervical lymph nodes.

Ocular manifestations are noted in all cases. Uveitis is the most important of the triad of prerequisites for a diagnosis of uveoparotitis. It is often the earliest symptom, and the resulting visual impairment is permanent, in varying degrees of severity. While the onset may be unilateral, with pain and dimness of vision, bilateral uveitis occurs almost invariably. The pupils may become dilated, irregular and fixed, with the tendency to development of posterior synechiae. Fatty deposits are seen on the posterior surface of the cornea. Nodules, definitely identi-

13. Mohn, A.: Ein Fall von Febris uveoparotidea (Heerfordt), Acta ophth. **11**:397, 1933.

14. Haferkorn, M.: Aspects of Heerfordt's Syndrome (Uveoparotid Fever), Monatschr. f. Kinderh. **63**:161, 1935.

fied as tuberculous, are said to occur in the iris. Less common findings include hemorrhages into the vitreous, optic neuritis, choroidoretinitis, keratitis, glaucoma, turbidity of the aqueous and cataract.

Facial palsy of the peripheral type is the commonest neurologic manifestation, occurring in from one third to one half of all cases. It may be bilateral, although more frequently it is unilateral. Whether the palsy results from choking of the nerve by swelling of the parotid gland, which it usually, although not uniformly, follows, is an unsettled question. There was evidence in Mohn's case to place the site of the lesion between the geniculate ganglion and the branching of the chorda tympani, excluding pressure from a swollen parotid gland. Loss of the sense of taste and bilateral deafness of the central type have been reported. Disturbance of the innervation of the uvula and of the vocal cords is rare. Dysphagia and dryness of the mouth are common. Dysarthria has been attributed to dryness of the mouth by some and to muscular weakness following facial palsy by others.

Polydipsia and polyuria have been reported occasionally, with dilute but otherwise essentially normal urine. Levin said he felt that in his case the condition represented true diabetes insipidus.

Involvement of the spinal nerves may be manifested by a diminished tactile sense, reduction or abolition of the tendon reflexes, tenderness over the peripheral nerves, paresthesias and wrist or foot drop.

Unfortunately relatively few reports have been made regarding the cerebrospinal fluid. If an examination is mentioned, slight abnormalities or none at all have been recorded. There may be moderate pleocytosis (5 to 20 lymphocytes per cubic millimeter) and a slight increase in the total protein content, with curves for mastic precipitation which are either flat or nearly so.

The blood picture, if altered at all, includes a slight reduction in the hemoglobin content and in the number of erythrocytes, with moderate leukocytosis. In some cases eosinophilia occurs, with a usual maximum of 5 per cent, although extremes of 10 or 15 per cent have been noted.

The urine is generally normal.

The reaction to the tuberculin test was recorded in twenty-one of the sixty-six cases reviewed by Savin, with seven positive results. In the sixty-six cases the Wassermann reaction of the blood was mentioned in thirty-one instances—twenty-nine negative, two positive and two doubtful results.

REPORT OF CASES

Although Hamburger and Schaffer [3] reported a case of uveoparotid fever from the Johns Hopkins Hospital in 1928, attention was not drawn

to the possibility of other cases until 1933, when the diagnosis was proposed by a visiting physician from South Africa in the following case:

CASE 1.—P. P., a Negress aged 20, was seen in the medical clinic in the division for syphilitic patients on Feb. 18, 1933, when convalescent from an illness for which a diagnosis of polyneuritis had been made. There was no suggestion of tuberculosis in her past and family histories. In childhood she had mumps. Manifestations of secondary syphilis appeared early in 1932. In April, after eight injections of neoarsphenamine were given, uveitis developed. This was followed in May by headache, weakness, vague abdominal pains, swelling of one side of the face and pain on moving the jaw. Paresthesias of the extremities, with signs of diffuse polyneuritis, soon appeared, and hospitalization was necessary. The Wassermann reaction of the blood was negative. The cerebrospinal fluid and urine were entirely normal. There was an essentially normal blood picture, excepting for 4 per cent eosinophils.

No facial paralysis appeared, although the facial swelling and tenderness suggested parotitis. There was a slight irregular fever, with the temperature ranging up to 99.8 F. Although the original impression was arsenical polyneuritis following antisyphilitic treatment, the possibility of atypical uveoparotitis was considered.

Although a positive diagnosis of uveoparotitis could not be made, the case provided the stimulus for future observations.

CASE 2.—M. W., a Negress aged 21, was admitted to a medical ward of the Johns Hopkins Hospital on Nov. 25, 1933, complaining of headache, nausea, vomiting and vague abdominal pains of six weeks' duration. Her stepfather died of pulmonary tuberculosis, and a brother was a patient in a local hospital with active pulmonary tuberculosis. The patient had measles and pertussis in childhood. There was no history of mumps. She was married at the age of 18 and shortly afterward had a cutaneous eruption, with a positive Wassermann reaction. A practitioner gave her a single intravenous injection. Her husband was known to be under treatment for latent syphilis in this clinic.

In August 1933 her illness began with malaise, fatigability, slight edema of the ankles and occasional bilateral earache. One month later bilateral parotid swelling occurred and progressed until the glands were "even with the ears." The swellings disappeared within a week. The patient gave no history of contact with mumps. Within two weeks headache in the frontal region, anorexia, nausea, vomiting and abdominal pains began. A pain in the chest was diagnosed as due to pleurisy. Two weeks later weakness of the left side of the face was observed, followed by marked thirst, an increased intake of fluid and an increased output of urine. By November difficulty in swallowing, hoarseness and regurgitation of fluids through the nose had become marked. The patient began to experience "pins and needles" sensations over the entire right side of the body. Two days before her admission to the hospital weakness to the point of prostration and stiffness of the neck were noted.

Physical Examination.—The temperature was 101.6 F., the pulse rate 130 and the respiratory rate 30 per minute. There was extreme weakness, with signs of dehydration and loss of weight. The neck was stiff. The pupils were dilated and equal and reacted sluggishly. No extra-ocular paralysis was found. The retinal veins were engorged. All the parotid swelling had disappeared, but most of the cervical lymph nodes were enlarged. The gums were bleeding. Examination revealed harsh breath sounds and persistent râles in the upper lobe of the right lung. Cardiovascular examination showed only tachycardia and a blood pressure

of 150 systolic and 100 diastolic. Diffuse abdominal tenderness was noted. In addition to stiffness of the neck, the neurologic examination revealed complete palsy of the left side of the face of peripheral type, paralysis of the left side of the uvula, unequal knee jerks, bilateral ankle clonus, a questionable Kernig sign and no Babinski sign. Encephalitis and acute syphilitic meningitis were strongly suspected.

Laboratory Examination.—The urine showed a specific gravity of 1.010, some albumin, no sugar, many hyaline casts and a few leukocytes and erythrocytes. These abnormalities disappeared gradually within three weeks.

Examination of the blood showed: hemoglobin, 10.8 Gm. per hundred cubic centimeters; erythrocytes, 3,400,000, and leukocytes, 10,000. The differential count was essentially normal. The leukocyte count varied from 10,000 to 14,000 for

A patient (case 2) with uveitis, weakness of the right side of the face and swelling of the parotid and lacrimal glands.

several weeks, with 3 per cent eosinophils. The Wassermann and flocculation tests gave positive results.

The cerebrospinal fluid was clear and colorless and under normal pressure. It was free from micro-organisms, as shown by smear and culture. There were 15 lymphocytes. The Wassermann reaction was negative with 0.2 cc. and doubtfully positive with from 0.4 to 1 cc. of fluid. The total protein content was 38 mg. per hundred cubic centimeters, the curve for the mastic precipitation was 0133100000 and the sugar content was 78 mg. per hundred cubic centimeters. Two weeks after the patient's admission to the hospital the cerebrospinal fluid gave a completely negative Wassermann reaction but showed 9 lymphocytes, a total protein content of 50 mg. per hundred cubic centimeters and a curve for mastic precipitation of 5554320000.

Chemical analysis showed essentially normal blood except that the non-protein nitrogen content was 39 mg. per hundred cubic centimeters shortly after the patient's admission to the hospital.

Roentgenologic studies showed that the sella turcica was normal. A small area of infiltration was seen in the upper lobe of the right lung when the patient was admitted and two weeks later areas of consolidation were noted in both hilar regions. These were interpreted as being due to tuberculous involvement. The reaction to the tuberculin test was negative with 0.01 and 0.1 mg. of old tuberculin intracutaneously administered.

Course.—After admission to the hospital the patient showed increasing stupor, and on the second day three generalized convulsions occurred. Delirium, hallucinations and a temperature up to 103 F. characterized this period. By the second week the blood pressure had fallen to a normal level, and the mental status was calm. Signs of involvement of the lower lobe of the right lung appeared. In the third week complete palsy of the right side of the face of peripheral type was present, while there was residual weakness of the left side. The reflexes were normal, but stiffness of the neck, with occasional nausea and vomiting, persisted.

In December, one month after the patient's entry, there was an increase in the intra-ocular tension (right eye, 55 mm. of mercury; left eye, 47 mm.—Schiötz). The media of the right eye were hazy, and the cornea became dull, the sclera injected and the eye painful. The visibility of the aqueous ray was increased in both eyes. Paracentesis of the anterior chambers was performed, followed by iridotasis. Slit lamp examination showed numerous fatty deposits on the anterior surface of the lens and on the posterior surface of the cornea of the left eye. Inoculations of guinea-pigs with the aqueous fluid gave negative results. The ophthalmologic diagnosis was bilateral uveitis and secondary glaucoma.

In January 1934 the sputum showed tubercle bacilli on five occasions. A long period followed with continued low grade fever, the temperature not exceeding 99.8 F. Physical and roentgenologic examinations showed extensive infiltration of the entire right lung, with thickening of the pleura. Five months after the patient's admission to the hospital there was a period of slight general improvement, and then the afternoon fever recurred, the temperature reaching a maximum of 101 F. The condition of the eyes improved, and examination in May 1934 showed a normal tension and no corneal deposits, with only postoperative pupillary defects and haziness of the media of the right eye. There was residual facial weakness on the right, and signs of infiltration of the right lung persisted.

Before tubercle bacilli were noted in the sputum, treatment was entirely symptomatic, except for the administration of iodides in considerable quantities and for half a dozen injections of a bismuth compound at odd times during the five months of hospitalization.

The patient was transferred to a sanatorium for tuberculous patients in June 1934, and her condition remained unchanged until October. She left of her own accord in November and reappeared at the Johns Hopkins Hospital, where she was reexamined at the clinic on December 13. She was obviously ill, and admission to a medical ward was requested, with the diagnosis of uveoparotitis.

It was found that a swelling of the parotid glands had recurred in October that was identical with that of the previous year. The right parotid gland became swollen first, followed a week later by swelling of the left gland. Both were hard and tender to pressure and were not adherent to the skin or underlying tissues. A daily elevation of temperature occurred. Although bilateral facial weakness

was observed at first, it became more marked on the left. The speech became mushy, and chewing was difficult. In November difficulty in closing the eyelids was noticed.

Physical Examination.—At the time of the patient's second admission to the hospital there was a temperature of 100 F., with slight acceleration of the pulse and respiratory rates. There was a scaly desquamation over the tibias. The parotid, lacrimal, sublingual and submaxillary glands, as well as the posterior cervical and axillary lymph nodes, were enlarged. Examination of the eyes showed injection of the conjunctivae and sclera, cloudiness of the anterior chamber and numerous mutton-fat deposits over the anterior capsule of the lens and the posterior surface of the cornea. The pupils were unequal, deformed and fixed. The fundi could not be seen clearly. The intra-ocular tension was normal. There was bilateral facial weakness, and the uvula was deviated to the left. Signs of infiltration of the right lung were present. No other general or neurologic abnormalities were detected.

Laboratory Examination.—The urine exhibited a trace of albumin and a few hyaline casts but became normal within two days.

The blood showed a normal erythrocyte count and hemoglobin content. The leukocytes numbered 9,000, with 69 per cent polymorphonuclear cells, 9 per cent being eosinophils. (Six weeks after the patient's admission to the hospital a maximum of 13 per cent eosinophils was noted, but for only a few days.) The Wassermann reaction of the blood was doubtful, but the flocculation test was positive.

The cerebrospinal fluid was clear, colorless and under normal pressure and contained 6 lymphocytes. The total protein content was 25 mg. per hundred cubic centimeters, and the curve for mastic precipitation was 333210000.

The sputum persistently showed tubercle bacilli. The reaction to the test with tuberculin, 0.1 mg. administered intracutaneously, was negative. Culture of catheterized secretion from the parotid gland yielded no growth. Electrocardiographic examination revealed no abnormality.

Course.—Small doses of neoarsphenamine were administered, but a generalized maculopapular rash with itching followed the third injection. This disappeared with cessation of treatment. Three weeks after the patient's admission to the hospital roentgen therapy was attempted for the parotid and lacrimal swelling. After the second treatment intense swelling and edema of the glands occurred, and roentgen therapy was abandoned. The sublingual tissues remained markedly edematous and resembled a false tongue for many weeks. Low grade fever persisted, the temperature averaging 99.6 F. After three months the lacrimal and parotid swellings had practically disappeared.

In June 1935 the essential findings were facial weakness on the right, enlargement of the sublingual glands, slight enlargement of the lacrimal gland, and signs of infiltration at the base of the right lung.

The patient was sent to the Tuberculosis Hospital of the Baltimore City Hospitals in September. Fever and tachycardia continued. Although signs suggestive of pleural effusion were noted at the base of the right lung, repeated thoracentesis revealed no fluid. The sputum persistently showed no tubercle bacilli. A course of neoarsphenamine was administered during a period of apparent general improvement. In January 1936 the patient's condition was increasingly unfavorable, and death occurred approximately two years and four months after the onset of the illness. Extreme weakness, hoarseness, edema of the ankles and bleeding by mouth marked the terminal illness. All efforts to obtain permission for autopsy were unsuccessful.

CASE 3.—M. P., a Negress aged 24, was seen in the Wilmer Ophthalmological Institute on July 31, 1934, complaining of headache and painful inflammation of the eyes of three weeks' duration. During that time there had been swelling of the eyelids each morning, and for one week she had experienced regurgitation of fluids through the nose, numbness of the face and an inability to smile. The speech had become mushy. For nearly a year there had been "nervous indigestion," with a loss of 20 pounds (9 Kg.). Her past history showed only pertussis and measles in childhood, and no suggestion of mumps, tuberculosis or syphilis could be elicited.

Physical Examination.—On August 9 the patient became obviously ill and was admitted to a medical ward of the Johns Hopkins Hospital. The temperature was 99.6 F., the pulse rate 96 and the respiratory rate 20. Although very restless, the patient was alert and cooperative. No cutaneous lesions were visible. Although no parotid enlargement was detected, the patient insisted that the right side of her face was fuller than usual. The lymph nodes at the angles of the jaw were palpable. Examination of the eyes showed a normal intra-ocular tension, pericorneal injection and irregularity of the left pupil, with posterior synechiae. The slit lamp revealed a strongly positive aqueous ray, with numerous amorphous deposits in the endothelial layer of the cornea; a muddy, edematous iris, with deposits of pigment over the anterior capsule of the lens of the left eye. Vision in the right eye was 20/70 and in the left eye 20/200 uncorrected. The ophthalmologist made a tentative diagnosis of acute uveitis, possibly due to syphilis. There was slight tenderness on pressure near the right ear in the parotid region.

Examination of the heart, lungs and abdomen showed no abnormalities. Neurologic examination revealed impairment of sensation on both sides of the face and neck; absence of the corneal reflexes; bilateral facial weakness, more marked on the right; poor movement of the uvula, and absence of the knee and ankle jerks. The diagnoses suggested were meningitis or bulbar palsy, with uveitis, due to syphilis.

Laboratory Examination.—The blood picture was not remarkable. The leukocytes numbered 9,000, with 1 per cent eosinophils. The Wassermann test of the blood was negative, but the flocculation test was positive. The cerebrospinal fluid was clear and colorless and under normal pressure. There were 10 lymphocytes, and the total protein content was 37 mg. per hundred cubic centimeters. The curve for mastic precipitation was 0011110000. The Wassermann reaction was negative. Five days later an examination of the cerebrospinal fluid gave the same results, except for a lymphocyte count of only 3 cells per cubic millimeter. The urine was normal. The reaction to the tuberculin test was negative (old tuberculin, 0.001 and 0.01 mg. intracutaneously). Roentgenologic studies showed signs of old mediastinal tuberculosis.

Because of uveitis associated with tenderness and questionable swelling in the region of the parotid glands, involvement of the cranial nerves, reduction of the deep tendon reflexes and fever, uveoparotitis was considered as a possible diagnosis.

Course.—Shortly after the patient's admission to the hospital the temperature reached a maximum of 102 F. but soon fell to a maintained average of 99.5 F. Four small injections of neoarsphenamine were administered, together with potassium iodide by mouth; salicylates and atropine were employed for the uveitis. By the time of the patient's discharge, on August 27, there had been a general improvement, although traces of uveitis, absence of the corneal reflexes and facial weakness persisted.

The patient was followed in the syphilis division of the medical clinic and in the outpatient department of the Wilmer Ophthalmological Institute. Anti-

syphilitic treatment was continued with arsenicals and bismuth for more than a year. While the ophthalmologists felt that syphilis was probably responsible for the uveitis, the staff of the syphilis division held the opposite opinion. By the end of 1934 only facial weakness and chronic uveitis were present. Throughout 1935 low grade uveitis persisted, and paracentesis of the aqueous of the right eye became necessary on two occasions. Deep marginal invasion of the cornea was suggestive of a tuberculous etiology, although there was relative insensitivity to tuberculin. Periods of improvement were apparently associated with arsenical therapy, but the possibility of the nonspecific reaction of arsenicals in uveitis (Stokes and O'Leary) was kept in mind. In spite of fairly regular antisyphilitic treatment, frequent exacerbations of the uveitis occurred during the last period of observation in 1936.

CASE 4.—B. S., a Negress aged 31, was seen in the Wilmer Ophthalmological Institute on March 27, 1935, complaining of painful sore eyes and facial weakness, with headache in the frontal region of one month's duration. There had also been anorexia, nausea and vomiting. The right side of the face became paralyzed on March 26, and fainting occurred once on that date. She gave a history of mumps, whooping cough, measles and chickenpox in childhood, but nothing suggestive of tuberculosis or syphilis could be elicited.

Physical Examination.—The patient was admitted immediately to a medical ward of the Johns Hopkins Hospital. Examination showed that the temperature, pulse and respirations were normal. No cutaneous eruption was seen. The axillary and inguinal lymph nodes were palpable. The parotid glands were not enlarged. Examination of the eyes showed a normal intra-ocular tension. With the slit lamp numerous deposits typical of punctate keratitis were observed on Descemet's membrane and in the anterior capsule of the lens of both eyes. The pupils were irregular and did not react well to light. Posterior synechia also was noted. Numerous opacities of the vitreous were seen. The fundi were normal. The only other abnormalities were found on neurologic examination. These consisted of complete peripheral facial palsy on the right, with a loss of the sense of taste on the anterior two thirds of the tongue, and sluggishness of the abdominal reflexes. The diagnostic impression was uveitis, with meningitis (?), probably syphilitic in origin.

Laboratory Examination.—The blood was normal, but the Wassermann reaction was positive. The cerebrospinal fluid and urine were entirely normal. No tuberculin test or roentgenograms were made.

Course.—Routine antisyphilitic treatment was begun. The patient was discharged, improved, with residua of the iritis on April 13. She was followed in the syphilis division, and the Wassermann reaction became negative within a few weeks. Treatment was pursued until January 1936, after which the patient was never seen.

CASE 5.—E. A., a Negress aged 28, was admitted to a medical ward of the Johns Hopkins Hospital on July 8, 1935. She complained of swelling of the face, difficulty in swallowing and regurgitation of liquids through the nose. The past history was unimportant, except for an illness diagnosed as pleurisy when she was 16 years old. There was no history of mumps. The illness had been present for seven weeks, with anorexia, eructation after meals, vertigo and malaise. This was followed by simultaneous swelling of both sides of the face, with aching and soreness of the throat. Vomiting occurred frequently. One week before the patient's admission to the hospital there developed hoarseness, a nasal voice,

dysphagia and regurgitation of fluids through the nose when an attempt was made to swallow. During the illness 12 pounds (5.5 Kg.) of weight was lost.

Physical Examination.—Examination showed that the temperature was 101 F., the pulse rate 110 and the respiratory rate 24 per minute. Weakness was extreme, and there was evidence of dehydration. No cutaneous eruption or enlargement of the lymph nodes was found. The parotid glands were enlarged symmetrically and were firm but not tender. There was no attachment to the skin or underlying tissues. Examination of the eyes showed abnormalities only in the right eye, in which vision was limited to counting fingers at 1 foot (30 cm.). Abnormalities were noted only on ophthalmoscopic examination. The margins of the disks were blurred, with indistinct cupping and marked neuroretinal edema. Several small, round spots of grayish exudate were seen in the region of the macula, and there were areas of old chorioretinal atrophy. The veins were full and tortuous, the arteries were narrowed and arteriovenous compression was marked. While the exudate was considered to be suggestive of tuberculosis, certain perivascular changes were thought to be characteristic of syphilis.

The remainder of the physical examination gave essentially normal results, except for the neurologic findings. These consisted of complete peripheral facial palsy, with weakness of the uvula and uveal cord, on the right, and of hypo-active deep tendon reflexes.

Laboratory Examination.—The blood picture was essentially normal. The Wassermann reaction was doubtful on repeated tests. The cerebrospinal fluid was clear and colorless and was under normal pressure. It showed 8 lymphocytes and a normal total protein content. The Wassermann reaction was positive with 0.4 cc., and the curve for mastic precipitation was entirely negative. The sputum repeatedly showed no acid-fast bacilli. The reaction to the tuberculin test was positive with 1 mg. of old tuberculin administered intracutaneously, but it was negative with smaller amounts. Roentgenologic studies showed markedly accentuated shadows of the roots of the lungs, with thickening of the pleura, which were interpreted as having a tuberculous origin. The skull and sella were normal. Gastro-intestinal studies showed no abnormalities.

Course.—The temperature ranged from 101 to 102 F. for two weeks. It was discovered that the odors of tobacco and ammonia could not be detected. The parotid swelling, facial weakness and hoarseness disappeared by the third week, and a few days later no abnormalities existed other than the anosmia and the changes in the right fundus. Five weeks after the patient's admission to the hospital examination revealed definite deposits on Descemet's membrane, with an increase in the visibility of the aqueous ray, in both eyes. The right disk was blurred and edematous. Vision in the right eye was 10/120, and that in the left eye was 20/30 at that time. Treatment, other than symptomatic, consisted of weekly injections of neoarsphenamine.

The patient was discharged to the care of the syphilis division on August 31, and antisyphilitic treatment of the routine type was continued. Serologic tests were negative in October, and the ocular condition was quiescent. In December an indurated erythematous eruption appeared on the legs, and this was diagnosed as erythema nodosum. Roentgenologic studies showed an increase in the shadows of the roots of the lungs. The ocular status was quiescent, and at the last observation early in the spring of 1936 no remarkable changes were noted in the general condition of the patient.

CASE 6.—M. F. J., a Negress aged 32, a native of North Carolina, came to the medical clinic of the Johns Hopkins Hospital on Aug. 23, 1935, complaining of

malaise and weakness of more than a year's duration. The past and family histories revealed nothing of importance before the onset of her complaints.

Beginning in February 1934 the patient had vague abdominal pain, malaise and weakness, with amenorrhea for three months. In November the right parotid and submaxillary glands were swollen, and this was followed quickly by severe headache, stiffness of the neck, weakness of the right side of the face and inability to swallow solids. These difficulties disappeared within two weeks, except for residual facial weakness and marked lassitude, accompanied with an afternoon temperature of 99 or 100 F. during the autumn. She was studied at the Duke University Hospital, Durham, N. C., in February 1935, where the findings consisted of "arrested pulmonary tuberculosis, secondary anemia, residual Bell's palsy on the right and iridocyclitis." Roentgenologic studies of the chest in that clinic revealed several enlarged rounded areas which were regarded as enlarged hilar nodes. A supraclavicular node was removed, and the biopsy report by Dr. Roger Baker, associate professor of pathology, described "an epithelioid cell reaction, probably tuberculous." No tubercle bacilli were observed in the sections.

Physical Examination.—The patient was examined at the University of Pennsylvania Hospital in April 1935. At that time there were undernutrition, low grade fever, enlarged cervical and supraclavicular nodes, anisocoria with posterior synechia, residual peripheral facial palsy on the right and enlarged hilar nodes, which were thought to be tuberculous. The uveitis was considered to be of tuberculous origin. The blood, cerebrospinal fluid, sputum and urine were entirely normal. Sections of the lymph node (previously made at the Duke University Hospital) were interpreted as showing "chronic adenitis very suggestive of tuberculosis."

The patient experienced numbness in the right arm and pain in the left side of the chest and had an unproductive cough, with anorexia, in the summer of 1935. Examination at the medical clinic of the Johns Hopkins Hospital on August 23 showed the temperature to be 99.4 F., with slight tachycardia. The glands at the angles of the jaw were enlarged slightly. There was old bilateral uveitis. With the slit lamp numerous mutton-fat deposits could be seen on the posterior surface of the corneas. There was increased retromanubrial dulness, and it was impossible to obtain a blood pressure reading in the right arm. Residual facial paralysis on the right constituted the remaining abnormality.

Laboratory Examination.—Serologic tests for syphilis were negative. The blood and urine were normal. The reaction to the tuberculin test was positive with 1 mg. of old tuberculin injected intracutaneously. Roentgenologic studies confirmed those previously described.

Course.—Roentgen treatment was undertaken for the mediastinal mass, with no visible change. Tuberculin therapy was begun, and the patient was returned to her home to continue the course of treatment. In May 1936 her physician stated that no appreciable change had occurred in the general condition or in the roentgenologic picture of the chest following a long course of tuberculin therapy.

COMMENT

The cases of six young Negresses are presented who showed a syndrome suggestive of or identical with that described as uveoparotitis, or uveoparotid fever. In four there was evidence of tuberculous infection. In five syphilis was evidenced by the history, physical findings or posi-

tive results of serologic tests. The diagnosis of acute syphilitic meningitis was proposed for three during the acute stage of the illness.

In no instance did examination of the cerebrospinal fluid support the initial impression of acute syphilitic meningitis, and in only one instance was there evidence of invasion of the central nervous system by syphilis. Such abnormalities as were discovered consisted of slight pleocytosis and a mild increase in the total protein content.

Parotitis was an outstanding finding in three of the patients and in case 2 a recurrence was an unusual feature. In two cases definite parotitis was questionable, and in one it was not observed. The type of parotitis seen in these cases corresponded closely with that described in the literature as occurring in uveoparotitis. With regard to the possibility of syphilitic etiology, Kemp and Moore [15] found involvement of the salivary glands in less than 0.07 per cent of the syphilitic patients at the Johns Hopkins Hospital.

Syphilitic parotitis [16] may be of three types: In early syphilis the gland swells rapidly and is tender to the touch and firm, with a tense, tender, reddish surface. The swelling is usually bilateral but not symmetrical. Suppuration occasionally occurs in the neighboring lymph nodes. The mass is closely adherent to the underlying tissues. Salivation, pain and dysphagia are prominent. Fever is rare. The infection may last for from two to twelve months.

In late syphilis parotitis develops insidiously. The tumor mass becomes attached to the underlying tissues and the skin. Fluctuation develops, with the appearance eventually of a gummatous ulcer. In congenital syphilis the lesion in the parotid gland is commonly a diffuse fibrosis. The gummatous type of lesion responds well to antisyphilitic treatment.

In two of the cases reported here it was possible to observe the character and course of the parotitis, which in these instances was unlike a syphilitic process. In two other cases parotitis was mild and transient, if existent, and in another case it was mild and of short duration.

The etiology of the ocular lesions is a most interesting question. In cases 2 and 6 the ophthalmologists were of the opinion that tuberculosis was the causative factor. Case 3 was the subject of considerable controversy, with arguments both for and against syphilis and tuberculosis. In case 5 the lesion was neuroretinitis, which has been described as occurring infrequently in uveoparotitis. There was lack of evidence of tuberculosis and improvement of the iritis with antisyphilitic treat-

15. Kemp, J. E., and Moore, J. E.: Syphilis of the Salivary Glands, Arch. Dermat. & Syph. **6**:57 (July) 1922.

16. Chargin, L., and Rosenthal, T.: Syphilitic Parotitis, Arch. Dermat. & Syph. **24**:236 (Aug.) 1931.

ment in case 4. No adequate data relating to the possible tuberculous etiology of the ocular manifestations were available in case 1.

Fever was present in five of the six cases presented here and was of many months' duration in two of these cases.

Facial paralysis occurred in five of the cases. Additional neurologic abnormalities have been described in the individual case reports. The first case of the series, in which the condition was characterized by uveitis and diffuse polyneuritis, must be regarded as unusual or as representing a forme fruste, if it is included as a case of uveoparotitis. The possibility of arsenical polyneuritis cannot be overlooked.

A review of the literature reveals a multiplicity of causes to which uveoparotitis has been ascribed, including tuberculosis, syphilis, beriberi, mumps, diphtheria, oral sepsis and "pseudoleukemia." It has been designated also as a variety of the Mikulicz syndrome. Most of these theories have been discarded, leaving two schools of thought. The question now stands as to whether the established clinical entity is due to tuberculosis or to some undiscovered infectious agent such as a virus. Garland and Thomson have advanced considerable evidence in favor of tuberculosis. This includes postmortem observation of miliary tubercles (four cases), biopsy of the iris (four cases), bulbar conjunctiva (one case), focal reaction to tuberculin (two cases) and pulmonary tuberculosis with positive findings in the sputum (one case). These authors declared in 1933 that they had found incontrovertible evidence of tuberculosis in more than one third of the recorded cases.

It is true that it has been impossible to demonstrate tubercle bacilli in biopsy sections of the parotid, lymphoid and ocular tissues, although such sections commonly show histologic evidence of a fibrosing endothelioid lesion that is highly suggestive of tuberculosis. Cavard injected such material intraperitoneally into guinea-pigs, producing fatal generalized tuberculosis in one instance. Collateral evidence of tuberculosis is more common in the form of the family history, pleural effusion, roentgenologic evidence, cervical adenitis and the reaction to tuberculin injected intracutaneously.

It is singular that five of the six patients reported on in this paper showed evidence of syphilis by serologic reaction, in the light of the fact that this infection has been recorded rarely in the cases of uveoparotitis previously reported. As far as can be determined, all previously reported cases have been in the white race, whereas this group is composed entirely of young Negresses. Thirty or 40 per cent of an unselected number of Negro hospital patients in this locality will show positive results of serologic tests for syphilis. The incidence of tuberculosis in Negroes of this city also is high. Syphilis is no longer

considered seriously as an etiologic agent in uveoparotitis, and in the present group of cases the infection is considered as being entirely coincidental.

As stated previously, the first case of uveoparotitis in this hospital was reported by Hamburger and Schaffer in 1928. At that time the authors gave as their opinion that the condition represented a form of the Mikulicz syndrome. They were unable to discover evidence of tuberculosis at that time. They have recently granted me permission to state that in their patient renal tuberculosis subsequently developed, for which nephrectomy was performed.

In conclusion, it appears that the more fully cases are investigated, the more evidence one finds in favor of tuberculosis.[17] Garland and Thomson seemed to be justified in designating the syndrome as uveoparotid tuberculosis, and the burden of proof is on those who favor an unknown virus as the causative agent.

SUMMARY

Six cases of patients presenting the syndrome of uveoparotitis, or uveoparotid fever, all young Negresses, are reported.

Five of these patients had syphilis and four tuberculosis. Three had both of these infections.

The weight of evidence favors the tuberculous etiology of uveoparotitis.

NOTE.—M. P. (case 3) was seen on Nov. 18, 1936, and she complained that she had suffered from a severe cough for seven weeks. There had been no loss of weight, and the temperature had been normal. Physical and roentgenologic examination showed evidence of infiltration of the upper lobe of the right lung suggestive of pulmonary tuberculosis. Examination of the sputum revealed no tubercle bacilli.

17. Uveo-Parotid Tuberculosis, editorial, Lancet **2**:763, 1934.

RELATION OF EXPERIMENTAL ATHEROSCLEROSIS TO DIETS RICH IN VEGETABLE PROTEIN

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In 1920 Newburgh and Squier [1] reported the occurrence of atherosclerosis in rabbits fed diets rich in animal protein. The diets used were of two kinds: one was a mixture of milk, scraped carrot and water to which casein was added; the other was a mixture of dried powdered beef and bread flour in the proportion 1:2. Although it appeared that the vascular lesions had been caused by the protein of the diets, such a conclusion was thought not to be warranted at that time because of the smallness of the group of experimental animals.

Later, investigation by Newburgh and Clarkson [2] showed conclusively that ingestion of muscle meat by rabbits results in extensive atherosclerosis of the aorta and other large arteries. Eight of eleven animals that were fed a diet containing 27 per cent protein derived chiefly from dried ground beef muscle for more than six months became atherosclerotic. In all the rabbits that were fed a diet containing 36 per cent protein derived chiefly from beef muscle for from eighteen to thirty-six weeks marked and extensive atherosclerosis developed. The occurrence and extent of the sclerosis were roughly proportional to the duration of the feeding of meat.

Since it had been shown by others [3] that feeding of cholesterol causes atherosclerosis in rabbits, it was suggested by some that the cholesterol contained in the meat diets caused the vascular lesions. Clarkson and Newburgh [4] showed, however, by the addition of different amounts of

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1. Newburgh, L. H., and Squier, T. L.: High Protein Diets and Arteriosclerosis in Rabbits: A Preliminary Report, Arch. Int. Med. **26**:38 (July) 1920.

2. Newburgh, L. H., and Clarkson, S.: The Production of Atherosclerosis in Rabbits by Feeding Diets Rich in Meat, Arch. Int. Med. **31**:653 (May) 1923.

3. Anitschkow, N., and Chalatow, S.: Ueber experimentelle Cholesterinsteatose, Centralbl. f. allg. Path. u. path. Anat. **24**:1, 1913. Wacker, L., and Hueck, W.: Ueber experimentelle Atherosklerose, München. med. Wchnschr. **60**:2097, 1913. Kon, J.: Experimentelle Atherosklerose, Tr. Jap. Path. Soc. **3**:8, 1913. Bailey, C. H.: Atheroma and Other Lesions Produced in Rabbits by Cholesterol Feeding, J. Exper. Med. **23**:69, 1916.

4. Clarkson, S., and Newburgh, L. H.: The Relation Between Atherosclerosis and Ingested Cholesterol in the Rabbit, J. Exper. Med. **43**:595, 1926.

cholesterol to normal diets, that the amount of cholesterol contained in the diets high in beef protein was too small to produce by itself hypercholesteremia or atherosclerosis. It was necessary to add ten times the amount of cholesterol found in the meat diets before atherosclerosis resulted. In this same investigation it was pointed out that animals in which atherosclerosis developed as a result of ingestion of beef muscle had, in every case, hypercholesteremia. This finding suggested, therefore, that the hypercholesteremia resulted from cholesterol synthesis within the body, caused in some way by the ingestion of diets high in meat, and that the vascular lesions resulted from the hypercholesteremia.

In order to investigate this problem further the following experiments were performed. It was desired to learn whether atherosclerosis would result from feeding diets rich in vegetable protein as it did from feeding muscle meat and, if it would, whether hypercholesteremia preceded the appearance of the vascular lesions.

EXPERIMENTAL METHOD

Three diets containing different amounts of vegetable protein were prepared as follows:

Diet 1	Gm.
Ground whole soy bean	600
Corn starch	1,000
Alfalfa meal	400
Sodium chloride	12

Diet 2	Gm.
Ground whole soy bean	1,200
Gluten flour	400
Alfalfa meal	400
Sodium chloride	12

Diet 3	Gm.
Gluten flour	1,600
Alfalfa meal	400
Sodium chloride	12

To each of these diets baking powder and water were added, and the mixture was kneaded into a dough and baked in an oven at 180 F. until a brown crust formed.

Samples of each diet (after baking) were analyzed for nitrogen by the Kjeldahl method. Diet 1 contained 2.3 per cent nitrogen, diet 2 5.8 per cent and diet 3 6.64 per cent. Using Jones'[5] factors for converting percentages of nitrogen

5. Jones, D. B.: Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins, U. S. Dept. Agric., circular 183, 1931, p. 6.

in soy beans and wheat flour into percentages of proteins,[6] the protein of each diet was calculated to be:

	Protein, %
Diet 1	13.1
Diet 2	33.0
Diet 3	37.8

It should be noted that these diets contained no animal food; consequently they contained no cholesterol. Schönheimer[7] has shown that plant sterols (phytosterol) are absorbed in infinitesimal amounts, if at all. These diets are therefore "metabolically" sterol free, so that any abnormality that might result from feeding such diets could not be ascribed to dietary sterol.

Eighteen rabbits approximately 4 months old were selected for this study. These animals were kept in individual cages. They were divided into three equal groups, each group receiving a different diet. Twice weekly the diet was supplemented by a feeding of lettuce or carrots. In order to avoid a deficiency of vitamin D, the rabbits were given ultraviolet radiation, each animal receiving the equivalent of approximately 1 human skin erythema dose weekly.

At intervals the animals were weighed and bled from the marginal vein of the ear. Five cubic centimeters of blood was removed on each occasion, clotting being prevented by the use of sodium oxalate. The whole oxalated blood was analyzed for its cholesterol content by the oxidative digitonin method of Okey.[8] After various lengths of time the animals of each group were killed, and the large arteries were examined grossly and microscopically for evidence of atherosclerosis.

RESULTS

All the animals fed diets 1 and 2, which were crumbly and had a pleasing taste, ate heartily from the outset and rapidly increased in weight; in fact, some became obese. The rabbits fed diet 3, which was hard and tasteless, ate sparingly during the early part of the study, during which time they either lost weight or grew only a little. After about three months they began to eat well and gained in weight. It will be shown later that these differences in the nutritional state of the animals are important.

The cholesterol values and autopsy observations are summarized in the accompanying tables.

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6. In soy beans the amount of nitrogen multiplied by 5.71 equals the amount of protein. In wheat the amount of nitrogen multiplied by 5.7 equals the amount of protein.

7. Schönheimer, R.: Ueber die Bedeutung der Pflanzensterine für den tierischen Organismus: Ueber die Sterine des Kaninchenkotes, Ztschr. f. physiol. Chem. **180**:32, 1929. Schönheimer, R.; von Behring, H.; Hummel, R., and Schindel, L.: Ueber die Bedeutung gesättigter Sterine im Organismus, ibid. **192**: 73, 1930.

8. Okey, R.: A Micromethod for the Estimation of Cholesterol by Oxidation of the Digitonide, J. Biol. Chem. **88**:367, 1930.

TABLE 1.—*Weight, Cholesterol Values of the Blood and Condition of the Aorta of Animals Fed Diet 1, Containing 13.1 Per Cent Vegetable Protein, Chiefly from Soy Beans*

Animal	Sex	Day of Experiment	Weight, Kg.	Blood Cholesterol, Mg. per 100 Cc.	Condition of Animal When Killed	Condition of Aorta
1	M	1	1.80	86	"Snuffles"	Normal
		48				
2	M	5	1.50	67		
		82	2.26	76		
		124	2.60	80	Healthy	Normal
3	M	38	2.68	74		
		138	3.70	64		
		192	3.70	119	Healthy	Normal
4	M	43	2.25	57		
		120	3.52	73		
		228	4.20	50	Healthy	Normal
5	M	1	1.88	72		
		43	1.97	69		
		120	2.35	85		
		228	2.85			
		281	2.93	67	Healthy	Normal
6	M	1	1.94	87		
		43	2.11	69		
		120	2.66	124		
		228	3.20	82		
		281	4.48	71	Healthy	Normal

TABLE 2.—*Weight, Cholesterol Values of the Blood and Condition of the Aorta of Animals Fed Diet 2, Containing 33 Per Cent Vegetable Protein, Chiefly from Soy Beans and Gluten Flour*

Animal	Sex	Day of Experiment	Weight, Kg.	Blood Cholesterol, Mg. per 100 Cc.	Condition of Animal When Killed	Condition of Aorta
11	M	1	2.00	87		
		38	2.40	89		
		99	2.80	91		
		159	3.15	84	Healthy	Normal
12	M	1	1.65			
		60	1.99	80		
		104	2.49	69		
		180	2.25	70		
		217	2.35	68	Bubo on neck	Normal
13	M	1	2.40	110		
		44	2.61	77		
		120	2.62	70		
		228	3.15	70	Healthy	Normal
14	M	1	1.60			
		31	2.00	98		
		92	2.75	85		
		168	3.07	67		
		276	4.10	54	Healthy	Normal
15	M	23	1.90			
		92	2.52	69		
		168		90		
		286	3.05			
		346	3.05	79	Healthy	Normal
16	M	1	2.27			
		60	2.42	86		
		104	2.90	85		
		180	2.90	80		
		288	3.50	67		
		340	3.15	134	"Snuffles"	Normal

None of the animals showed gross or microscopic evidence of atherosclerosis of the aorta or other large vessels. The level of cholesterol in the blood remained close to the preexperimental value in the majority of animals. Considering the range of 35 to 125 mg. per hundred cubic centimeters found by Clarkson and Newburgh [4] as normal, only rabbits 16, 21, 22, 23, 25 and 26 showed hypercholesteremia at any time. In none of the animals was the level of cholesterol in the blood very high; the highest value observed was 182 mg. per hundred cubic centimeters.

TABLE 3.—*Weight, Cholesterol Values of the Blood and Condition of the Aorta of Animals Fed Diet 3, Containing 37.8 Per Cent Vegetable Protein, Chiefly from Gluten Flour*

Animal	Sex	Day of Experiment	Weight, Kg.	Blood Cholesterol, Mg. per 100 Cc.	Condition of Animal When Killed	Condition of Aorta
21	F	23	1.16	89		
		147	2.20	170	Undernourished	Normal
22	M	1	2.50	65		
		28	2.10			
		35		97		
		96	1.95	182		
		156	2.36	70	Healthy	Normal
23	F	23	1.05	73		
		147	1.88	126		
		209	2.20	154	Slightly undernourished	Normal
24	M	1	1.90	72		
		43		87		
		104	2.10	69		
		164	2.33	95		
		258	2.43	65	Healthy	Normal
25	M	1	2.05	96		
		36	1.50			
		43		106		
		104	1.70	156		
		164	2.50	137		
		258	3.12	104		
		320	3.30	97	Healthy	Normal
26	M	1	2.50	93		
		28	1.80			
		96	1.97	127		
		156	2.13	93		
		280	2.65	74		
		342	2.65	110	Healthy	Normal

found on the ninety-sixth day in rabbit 22. In three animals (rabbits 22, 25 and 26) the slight elevations of the cholesterol content of the blood occurred at the times the animals were considerably undernourished. They later began to eat heartily of the same high protein diet; they gained weight, and the cholesterol content of the blood returned to normal.

Only three animals had hypercholesteremia when killed. After two hundred and eighty-eight days with a normal cholesterol value, rabbit 16 contracted "snuffles," and its weight decreased from 3.5 to 3.15 Kg.

during the two months just prior to its death, when the slightly high value of 134 mg. per hundred cubic centimeters was found. Rabbits 21 and 23 were younger and very small females when the feeding started. They increased in weight but were still undernourished when killed. The cholesterol values were 170 and 154 mg. per hundred cubic centimeters, respectively, when the animals were killed.

COMMENT

Since hypercholesteremia was in every case associated with undernutrition and since the cholesterol content of the blood returned to normal in every instance when a normal nutritional state again obtained, while the animals remained on the same high protein diets, the rare occurrence of hypercholesteremia evidently was not caused by the protein of the diets but resulted from malnutrition.

It is clearly shown that diets high in vegetable protein do not produce atherosclerosis. Diets 2 and 3 contained as much or more protein than did the beef muscle diets fed by Clarkson and Newburgh, which in every case caused atherosclerosis in from eighteen to thirty-six weeks. There are two possibilities which would explain the occurrence of atherosclerosis in rabbits fed on diets rich in animal protein and the absence of such vascular lesions in rabbits fed on diets high in vegetable protein: Either the animal protein itself when fed to rabbits causes this lesion, or there is a nonprotein constituent of these animal foods which is the etiologic agent.

The first of these explanations seems very unlikely. Complete analyses of the protein of beef muscle, gluten flour and soy beans are not available. Yet the analytic data that exist show the composition of these various proteins themselves to be such that the difference in results obtained cannot logically be attributed to differences in the proteins. It appears, then, that the factor responsible for the development of atherosclerosis is a nonprotein constituent of these animal foods. The diet used by Newburgh and Squier, which contained milk and casein, may well have contained enough cholesterol to account for the vascular lesions observed.[9] It must be remembered, however, that Clarkson and

9. In order to determine whether this was true, my colleagues and I wished to give a diet rich in casein and sterol free and observe whether atherosclerosis would result. We were unsuccessful in completely freeing casein of sterol by any method of extraction. It could be rendered very poor in sterol, however, by repeatedly extracting it in hot alcohol according to the method of Randles and Knudson (Studies on Cholesterol: Synthesis of Cholesterol in Animal Body, J. Biol. Chem. **66**:459, 1925). A diet was made up consisting of 40 per cent of this extracted casein and 60 per cent sucrose, supplemented by carotene, ether-extracted yeast extract and linoleic acid. Unfortunately, the rabbits would not eat this diet.

Newburgh showed that their beef muscle diets contained too little cholesterol to cause atherosclerosis. It appears, therefore, that there is a nonprotein constituent other than cholesterol in animal flesh which when ingested in sufficient quantities by rabbits causes hypercholesteremia and atherosclerosis. Further investigative studies of this problem are planned.

CONCLUSIONS

Diets containing 33 and 37.8 per cent of vegetable protein fed to rabbits for as long as eleven months failed to produce atherosclerosis.

Rarely were cholesterol values of the blood observed which were higher than the normal range. These were in every case associated with undernutrition and are thought not to result directly from ingestion of large quantities of vegetable protein.

These data suggest that the atherosclerosis observed when rabbits were fed diets rich in muscle meat was due to a nonprotein constituent of this animal food.

TORULA INFECTION OF THE CENTRAL NERVOUS SYSTEM

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Torula infection of the central nervous system is a rare disease but one which is of interest to the internist and the syphilologist as well as the neurologist. Clinically, in these cases a diagnosis of tumor or abscess of the brain, tuberculous meningitis, encephalitis or dementia paralytica is made, and frequently the true etiology is not revealed until autopsy is performed.

Rusk, in 1912, under the title "Systemic Oidiomycosis," was the first in this country to report a case of torula infection of the central nervous system. His report was antedated in Germany, however, by that of von Hansemann in 1905 and that of Türk in 1907, each of whom reported one case. It remained for Stoddard and Cutler in 1916 to describe the clinical characteristics of this disease and to isolate the causative organism by cultural methods, animal inoculations and histologic sections. They differentiated this condition from other mycotic infections of the central nervous system and named the organism Torula histolytica. They analyzed the previously reported cases and added reports of two of their own cases. By 1919 only thirteen instances of cerebral torulosis had been reported. Freeman and Weidman in 1923 gave a critical analysis of the cultural characteristics and classification of Torula. Freeman later reviewed the gross and histopathologic changes observed in nineteen cases of torulosis and in 1931, in an exhaustive monograph, he collected the reports of forty-three cases. Since then fifteen more instances have been recorded, in addition to the two cited here, making a total of sixty cases of invasion of the central nervous system by Torula (table 1) reported in the literature.

REPORT OF CASES

CASE 1.—A German aged 59, the superintendent of a foundry, was admitted to the medical service of the Cleveland City Hospital on Oct. 26, 1933. His illness began five and one-half years before his admission to the hospital, with headache, vomiting, blurring of the vision and weakness. During the six months after the onset of the illness he was hospitalized on four different occasions by

From the Department of Dermatology and Syphilology, Western Reserve University and the Cleveland City Hospital, Cleveland, service of Dr. Harold N. Cole.

TABLE 1.—*Analysis of Sixty Cases of Cerebrospinal Torulosis*

	No. of Cases
Sex of patient	
Males	39
Females	20
Not stated	1
Age distribution in years	
Under 10	1
10-19	6
20-29	9
30-39	9
40-49	15
50-59	16
60-69	2
Not stated	2
Diagnosis made before culture or autopsy	
Tuberculous meningitis	10
Meningitis (unqualified)	5
Encephalitis	7
Tumor of brain	6
Abscess of brain	4
Dementia psychosis	5
Dementia paralytica	2
Miscellaneous	2
Not stated	19
Duration of illness in months	
0-1	3
1	6
2	10
3	9
4	8
5	3
6	3
7	1
8	2
9	1
10-12	1
13-24	3
25-36	5
37-48	0
49-60	0
61-72	1
Unknown	4
Organs involved, including central nervous system	
Lungs	9
Generalized	8*
Kidney	2
Spleen	1
Adrenal glands	1
Abdominal node	1
Peribronchial node	1
Tonsil	3
Subcutaneous tissue	2
Skin	5
Central nervous system only	30
When diagnosed	
Post mortem	23
Ante mortem	37
How diagnosed	
Antemortem cultures only	10
Biopsy of brain ante mortem	2
Postmortem histologic sections	22
Cultures and postmortem histologic sections	26

* In one case the lungs were not involved.

various medical attendants. Despite extensive roentgenographic and laboratory investigation, the only findings were "300 cells in the spinal fluid, chiefly lymphocytes and some small monocytes of various sizes and staining depth. There was a positive reaction to the globulin test, and the colloidal gold curve suggested the presence of meningeal irritation. Guinea-pig inoculations gave negative results. The patient was discharged ambulatory, with a final diagnosis of meningitis unqualified." Except for an occasional remission, his condition grew progressively worse, and about one year prior to his entry to this hospital he was confined to his bed.

Physical examination revealed that the patient was apathetic and not acutely ill, showing evidence of a loss of weight. Except for moderate arteriosclerosis, weakness and general muscular atrophy, there were no definite findings. The fundi showed some grayish exudate covering the vessels as they emerged from the central portion of the nerve head as probable evidence of an old choked disk. The impression reported at that time was that the condition was tumor of the brain, cerebral thrombosis, tuberculous meningitis or cerebrospinal syphilis. Laboratory investigation, including culture of the spinal fluid, revealed no abnormality. Roentgenograms of the chest and skull showed no evidence of a pathologic process. Roentgenograms revealed the presence of destructive mastoiditis.

Dr. L. Karnosh, of the neurologic department, who was called in in consultation reported the following neurologic components: generalized hyperreflexia, with more lively reflexes on the right; absence of the superficial reflexes symmetrically; a strong Babinski reflex on the right; a reduction in the size of the right pupil, with fine nystagmus when the eyes were directed to the right; mild hypesthesia over the second and third divisions of the right trigeminal nerve; voluntary and mimetic movements of the right side of the face, due to weakness; some central deafness on the right; hemihypesthesia to all qualities of sensation on the left; astereognosis of the left hand, and a very unsteady gait, which was attributed to general weakness. Combined cisternal and lumbar puncture revealed a practically complete subarachnoid block. The initial manometric readings were 60 mm. of water in the cisterna and 0 mm. in the lumbar region. The Queckenstedt test showed a 20 mm. rise in the pressure in the cisterna and only 2 mm. in the lumbar region, while straining caused a 20 mm. rise cisternally and a 10 mm. rise in the lumbar region.

It was believed that "most of the patient's symptoms were due to a diffuse process about the brain stem, from the midbrain to the lower border of the pons. In coordinating such symptomatology with the findings in the spinal fluid and with the unusual character of the disease, it was assumed that the lesions were of a granulomatous type, and it was therefore suggested that the condition might be due to a blastomycotic infection." The patient was transferred to the dermatologic service for further investigation and mycologic study. Suitable methods of culture and animal inoculation established the diagnosis of torula invasion of the central nervous system.

During the patient's entire stay of four months in the hospital he was afebrile except for a brief period of fever six weeks after entry, due to the development of bilateral purulent otitis media. The headaches, vomiting, weakness and disorientation progressed, and he died on December 29, five and one-half years after the onset of the illness.

Laboratory Examination.—The blood count showed: hemoglobin, 80 per cent (Sahli); erythrocytes, 5,050,000; leukocytes, 9,700, and polymorphonuclears, 76 per cent. Chemical analysis of the blood showed: sugar during fasting, 92 mg.; cholesterol, 250 mg.; urea nitrogen, 12.1 mg.; total protein nitrogen, 1,000 mg.;

nonprotein nitrogen, 31 mg.; total protein, 6.05 mg.; albumin, 3.41 mg.; albumin-globulin ratio, 1.28, and chlorides, 402 mg. per hundred cubic centimeters. The icteric index was 6. The Wassermann reaction of the blood was negative, and cultures of the blood, including cultures for Torula, were sterile.

Repeated lumbar puncture showed the spinal fluid to be slightly xanthochromic. The cell count varied from 75 to 280, with a predominance of lymphocytes. An occasional yeast cell could be seen. Chemical analysis of the fluid revealed that the sugar content was markedly decreased, varying from 7 to 15 mg. per hundred cubic centimeters. The analysis also showed: sodium chloride, 612 mg.; non-

Fig. 1 (case 1).—Photomicrograph (low power) of the choroid plexus; hematoxylin and eosin stain. Note the destruction of the normal architecture and the replacement of tissue by pseudocysts.

protein nitrogen, 22 mg.; total nitrogen, 393 mg., and total protein, 2.32 Gm. The Wassermann reaction was negative, and the mastic curve showed an increase in the middle zone.

A complete autopsy was performed by Dr. H. S. Reichle, of the department of pathology. The final anatomic diagnosis was infectious granulomatosis (T. histolytica), with invasion of the meninges and brain; chronic leptomeningitis, with external and internal hydrocephalus; chronic purulent mastoiditis; pulmonary emphysema; severe anthracosis and bronchopneumonia in the lower lobes of both lungs.

Torula in pure culture was recovered from the spinal fluid beneath the pons. A careful cultural search did not reveal the organism in the tonsils lungs, bronchi, spleen, liver, kidney, extradural or subdural spaces or mastoid processes. Cultures of the heart blood were sterile. Except for the brain and meninges, histologic sections of all the organs failed to reveal the presence of Torula.

CASE 2.—A white man aged 41 was first seen by a physician in June 1933. He complained of headaches in the frontal region of four months' duration. Results of physical examination were essentially unimportant. The headaches became progressively worse, and four months later the patient was suffering from

Fig. 2 (case 1).—Photomicrograph (moderate magnification) of the choroid plexus; hematoxylin and eosin stain. Note the granulomatous inflammatory reaction with lymphocytes, plasma cells and giant cells. The organisms can be seen as small, round bodies surrounded by a clear space or "lytic zone." Many of these spaces have coalesced, forming cystlike areas containing the organisms.

nausea, vomiting and dizziness. A Wassermann test of the blood was negative. Lumbar puncture revealed no abnormality, only 3 cells being seen. Ophthalmoscopic and otorhinolaryngologic investigations revealed nothing of significance. Roentgenograms of the chest and skull were negative, except for sclerosis of the left mastoid process and obliteration of the air cells. A neurologist who was called in consultation made a diagnosis of tumor of the frontal lobe of the brain.

The patient continued to suffer from headaches and vomiting. Three weeks' hospitalization in November 1933, nine months after the onset of the illness, revealed no definite physical findings, except evidence of an early stage of choked disk. The pressure of the cerebrospinal fluid was 300 mm. There were 160 cells, mostly lymphocytes. The Pandy test gave a positive result, with an atypical colloidal gold curve. The Wassermann reaction was negative.

For lack of any definite etiologic information and in view of the possibility that the condition might be due to syphilis of the central nervous system, antisyphilitic therapy was instituted. Repeated spinal puncture alleviated the symptoms but revealed little variation from the findings of the earlier examinations. The eyegrounds were again examined, and it was decided to give the patient tryparsamide. He received five weekly injections, making a total of 9 Gm. Ten days after the last treatment rapid failure of vision resulted, with marked visual constriction. Headache and vomiting continued. An internist who was called in consultation favored the diagnosis of multiple sclerosis. In July 1934, seventeen months after the onset of the illness, because of the possibility of cerebral syphilis, the patient was referred to Dr. H. N. Cole, who made a tentative diagnosis of torulosis. The patient was hospitalized for lumbar puncture and further study.

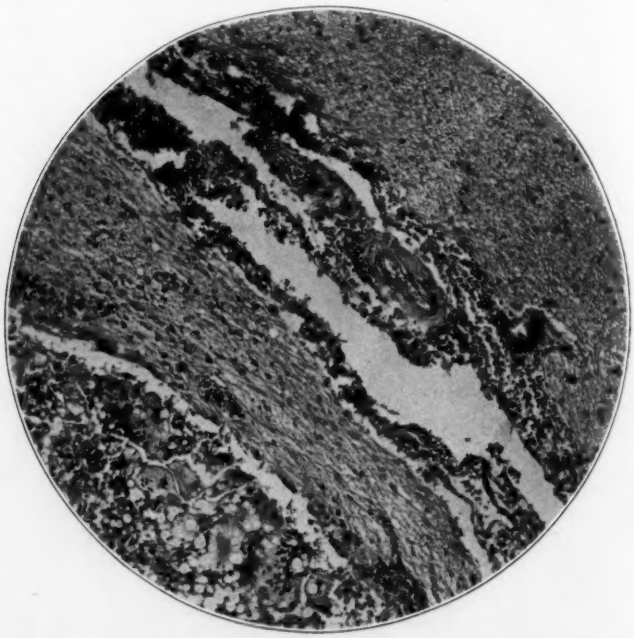

Fig. 3 (case 1).—Photomicrograph (moderate magnification) of a section of the brain; hematoxylin and eosin stain. Note the meningeal reaction about the pia-arachnoid of a cerebral sulcus, chiefly lymphocytes and plasma cells.

The same procedure as in the first case established a definite diagnosis of torula infection of the central nervous system by means of cultural studies and animal inoculations.

CLINICAL PICTURE AND DIAGNOSIS

The clinical manifestations of this disease are variable but can readily be understood when the increased intracranial pressure, the multiple granulomatous or cystic lesions and the involvement of the meninges are considered. The disease often sets in insidiously, with

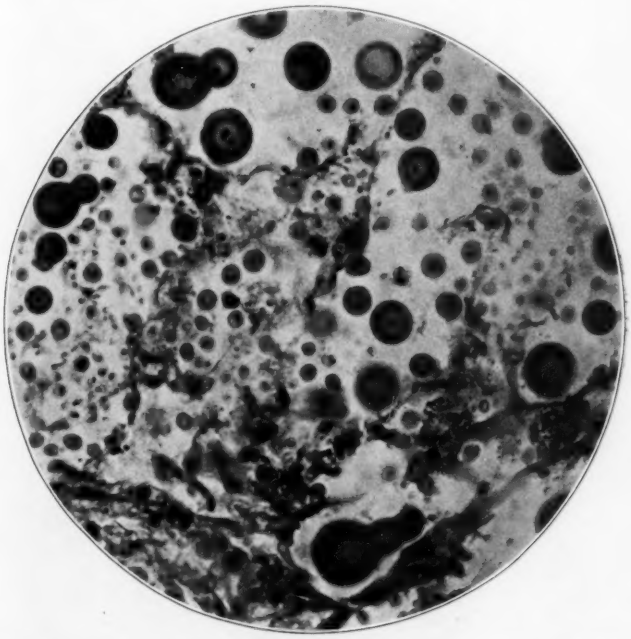

Fig. 4.—Photomicrograph (high power) of a section of the lung of a mouse; hematoyxlin and eosin stain. The round, budding organisms are clearly shown. Note the variation in size, capsule formation, sharp margins, granules and lack of inflammatory reaction.

intermittent severe headaches in the frontal region, later becoming more continuous, although occasionally the onset is violent and sudden, with excruciating headaches and vomiting. At times this cephalalgia may be the only clinical manifestation of the disease.

Dizziness, vertigo, stiffness and pain in the back of the neck are common symptoms. In many patients mental disorders are likely to occur, such as depression, disorientation, apathy, restlessness, irritability

and delirium. Amblyopia is common. Staggering, ataxia and hemiplegia have been noted. The commonest physical finding is stiffness of the neck, the Kernig sign, as well as the Brudzinski sign. The reflexes are variable. The patellar and the achilles tendon reflex are usually lost. Occasionally the Babinski sign is obtained. Neuroretinitis, papilledema and choked disk are of frequent occurrence. Other ocular disorders, such as strabismus, nystagmus and ptosis, as well as diplopia, also are noted.

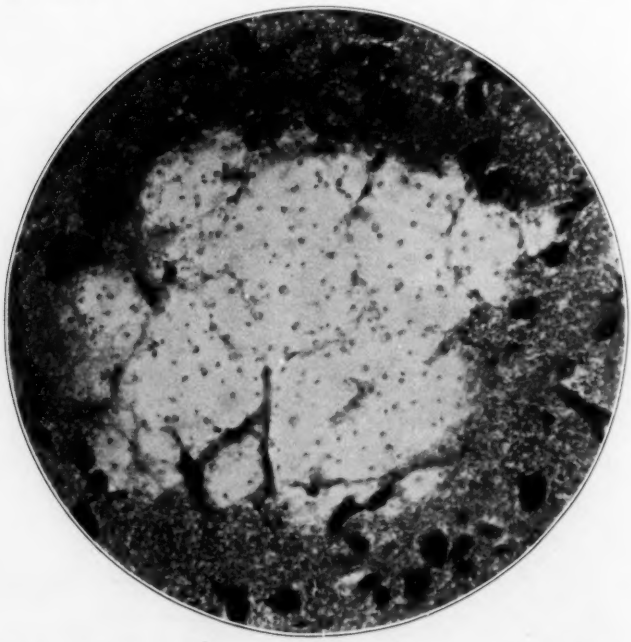

Fig. 5.—Photomicrograph (moderate magnification) of a section of the brain of a mouse; hematoxylin and eosin stain. Note the cystic lesion containing Torula and remnants of brain tissue as thin trabeculations extending from the margins within the cyst.

As a rule the patient is afebrile, and the symptoms of an acute infection are absent. Rarely is the temperature over 101 F. or the pulse rate over 100. The blood pressure remains normal. As the disease progresses, loss of weight and strength invariably occurs. The course is progressively downhill, although occasional periods of remission may be noted. The patient ultimately becomes comatose and dies of respiratory failure.

Torulosis of the central nervous system (table 1) is a disease of adult life, two thirds of the patients being between 30 and 60 years of age. The duration is variable, but in the majority of instances from one to six months of illness has been reported. Race, occupation and geography seem to be of no importance as regards incidence, for the disease has been noted in persons in all walks of life and in various parts of the globe. Males outnumber females two to one. A clinical diagnosis is most difficult because of the protean manifestations of this disease and its close simulation of more common conditions, particularly tuberculous meningitis, tumor or abscess of the brain and enceph-

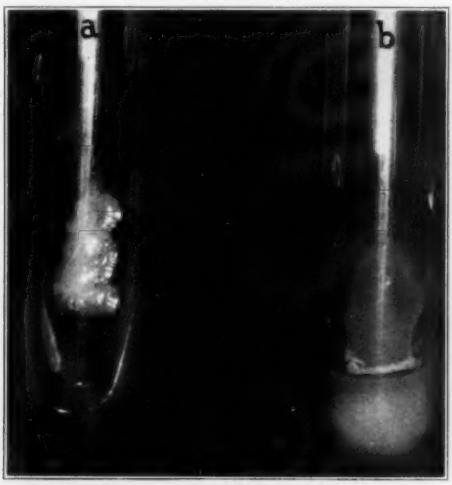

Fig. 6.—A month old culture of Torula from the second patient grown on Sabouraud's maltose agar medium (*a*). This shows the type of growth occasionally encountered—a moist, glistening, pasty, but rather firm growth which tends to from sectors about the periphery. A month old culture of Torula (courtesy of Dr. Weidman) grown on Sabouraud's maltose agar medium (*b*). This shows the typical mucoid, pasty, semifluid colony, with a major portion of the growth in the dependent portion of the tube.

alitis. Differentiation of torulosis from syphilis of the central nervous system is often necessary. However, the Wassermann reaction of the blood, the findings in the spinal fluid and/or a characteristic history invariably confirm the diagnosis in cases of syphilis. The differentiation of torulosis (table 2) from other mycotic infections of the central nervous system depends, of course, on cultures of the spinal fluid and perhaps on animal inoculation. In cases of cerebral involvement due to blastomycosis, coccidiosis, sporotrichosis or actinomycosis, invasion

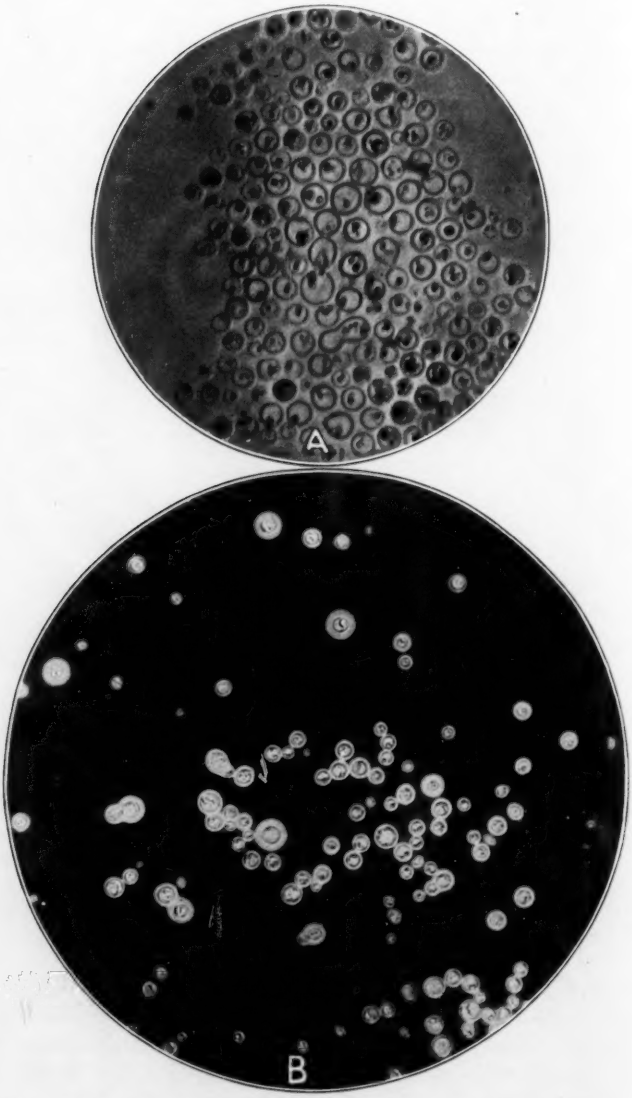

Fig. 7.—*A*, a hanging drop preparation of Torula in saline solution. Note the morphologic aspects and the various stages of budding. *B*, an india ink preparation of culture *a* shown in figure 6. The mucinoid envelop is seen as a clear zone or halo surrounding each organism.

TABLE 2.—*Differential Features of Yeast, Monilia, Torula, Blastomyces and Coccidioides* *

Genus	Synonyms or Name of Disease	Sugar Fermentation	Morphology in Lesions	Morphology in Culture	Cultural Characteristics	Animal Inoculation	Systems Involved	Pathologic Changes Produced
Saccharomyces	True yeasts	Strong	Oval and round budding cells, 5 to 15 microns in diameter; no mycelium (ascospores ?)	Oval and round budding cells, 5 to 15 microns in diameter; no mycelium; ascospores present	Round, pasty, cream-colored colonies	None	Skin; mild extent only	Abscess formation, with polymorphonucleosis, giant cells, necrosis and epithelial growth
Monilia	Oidium albicans	Moderate	Oval and round cells, 5 to 10 microns in diameter; mycelium usually present; no spores	Oval and round budding cells, 3 to 10 microns in diameter; mycelium usually present in old cultures; no spores	Round, pasty, glossy, raised, cream-colored colonies; marginal fringe when mycelium is present	Rabbits; intrapulmonary	Mucous membrane, mouth, gastrointestinal tract, lungs, intertrigenous portions of skin	Chronic ulceration
Torula	1. Wild yeast 2. Pseudo yeast 3. Cryptococcus hominis 4. European blastomycosis (Busse-Buschke disease)	Feeble	Encapsulated round and ovai, budding, thick-walled cells, usually 5 to 8 microns in diameter; occasionally 2 to 4 microns; no spores; no mycelium	Round and oval budding capsulated cells, 4 to 6 microns in diameter; no spores; no mycelium	At first, cream-colored; later yellow to brown; smooth, moist, mucoid colony	White mice and rats; intraperitoneal or intracerebral	Central nervous system chiefly; lung, viscera and skin to a lesser extent	Gelatinous yeast tumors, with slight inflammatory reaction, marked endothelial hyperplasia, foreign body giant cells, cysts in brain and occasional caseation
Blastomyces	1. Blastomyces dermatitidis Gilchristi 2. Oidiomycetes 3. Oidium dermatitidis 4. Chicago disease 5. American blastomycosis	None	Round, budding, thick-walled cells; 8 to 20 microns in diameter; no capsules; no spores; no mycelium	Mycelial growth; conidia and chlamydospores; raquet mycelium	Smooth, grayish growth; later aerial mycelium, white to tan, downy or fluffy colony; radial folds and concentric rings; sometimes spiny growth	White mice and rats; intraperitoneal or intratesticular	Skin always; often bones, lungs, viscera and central nervous system	Miliary abscesses, connective tissue overgrowth, caseation, necrosis, epithelial hyperplasia, tubercular nodules and ulcers; polymorphonuclears present; not gelatinous
Coccidioides immitis	1. Coccidioidal granuloma 2. California disease	None	Round, thick-walled cells, 5 to 75 microns in diameter; endospore formation; surface of cells often spiny; no budding; no mycelium	Mycelial growth; no conidia; intercalary chlamydospores; raquet mycelium	Smooth, grayish growth; later grayish white fluffy growth, occasionally spiny growth	Guinea-pigs; intraperitoneal	All organs, including central nervous system; skin often	Chronic granulomas with abscess formation, necrosis ulceration, giant cells, fibrosis and erosion due to endothelial hyperplasia

Compiled after various authors (Freeman, 1931; Henrici; Weidman; Benham).

of the nervous system is usually secondary to primary involvement elsewhere, particularly in the skin or subcutaneous tissue.

LABORATORY EXAMINATION

The routine laboratory examination of the blood and urine offers little as an aid in making a differential diagnosis. Mild anemia may be present, the leukocyte count is variable and there is no eosinophilia. Studies of the blood chemistry show no significant findings.

The examination of the spinal fluid, however, is most important. The fluid is clear, xanthochromic or turbid and under increased pressure. The cell count is increased, usually ranging from 200 to 800 cells, but there may be as few as 3 or as many as 1,000 cells. In many cases a pellicle forms. The specific gravity is usually about 1.008, and there is an increase in the albumin and globulin contents. The sugar content is variable but may be markedly decreased. The colloidal gold curve is not constant but is most often typical of meningitis or syphilis. The Wassermann reaction is negative.

Routine examination of the spinal fluid for a cell count in the counting chamber or a hanging drop preparation of the sediment from a centrifugated specimen may reveal the presence of the yeastlike cells, and even budding may be noted. A smear from this sediment may show round or oval, although somewhat distorted, gram-positive cells. A Wright stain (Jacobson) will help to distinguish lymphocytes from torulas, as the latter are not differentiated by this stain. If a loopful of the sediment is mixed with a drop of india ink on a slide and a cover slip is applied, the yeastlike organisms will appear to be surrounded by a halo and will stand out sharply against the black background. Microscopic examination alone, however, is inconclusive, and for a positive diagnosis culture and animal inoculation are essential.

ETIOLOGY

Torula is an oval or round, double-contoured, highly refractive yeastlike organism which reproduces both in tissue and in culture mediums only by budding. Sporulation and the formation of hyphae do not occur. It is widely distributed in nature and has been cultivated from plants and the bodies of many insects, as well as from canned butter (Henrici) and milk. It is ordinarily nonpathogenic. Various saprophytic or avirulent strains have been recovered also from normal skin (Benham and Hopkins), from the throat [1] and from the gastro-intestinal tract of man (Anderson).

Certain strains, however, have pathogenic properties (Benham) and become parasitic on man. Cases of torulosis in animals also have been

1. Tanner and Lampert. Tanner, Lampert and Lampert.

reported.[2] The portal of entry is not known, but the organisms gain entrance most likely by way of the upper respiratory tract to the lung and the central nervous system. While Torula has a special predilection for nerve tissue, generalization may occur. Rarely, the lungs only may be involved.[3] At times localized abscesses [4] and cutaneous lesions in conjunction with cerebral involvement [5] have been noted. However, nonfatal localized lesions without cerebral involvement also have been reported.[6] In some instances the relation of Hodgkin's disease to torulosis has been demonstrated.[7]

An antemortem diagnosis can be made only by direct examination of the spinal fluid, corroborated by cultural studies and animal inoculations. Repeated examination of the spinal fluid may be necessary, as at times the organisms may be few. Centrifugation and examination of the sediment will assist materially.

Torula appears to grow readily on the ordinary bacteriologic mediums, although an acid medium, such as Sabouraud's maltose agar, favors a more rapid growth. Sufficient time, however, should be allowed for the colonies to appear. The tubes should not be discarded for at least ten days. The cultures grow equally well at room temperature and in the incubator. Fresh spinal fluid is not a requisite, as the organisms are resistant. The colonies first appear as minute, elevated, whitish, circular, glistening dots with well defined margins. They grow slowly and are of a moist and pasty consistency, so that they tend to slide down the slant to the dependent portion of the tube. Primary cultures usually appear within four or five days, although subsequent transplants grow much more rapidly. The cultures tend to become yellowish brown with age. Young cultures are gram-positive, while older cultures are gram-negative.

In broth the growth collects in the bottom in a compact mass without turbidity of the medium. Microscopic cultures or a hanging drop preparation from a gross culture will not show any spore formation or hyphae. In one month old colonies an india ink preparation (Weidman and Freeman) will show the organism's most characteristic feature —a clear zone or halo surrounding each cell. The ability of this organism to ferment sugars is very feeble (Weidman).

For animal inoculation, white mice are best. One cubic centimeter of the organisms suspended in saline solution is injected intraperito-

2. Frothingham. Weidman and Ratcliffe.
3. Sheppe. Hardaway and Crawford.
4. Smith and Crawford. Johns and Attaway.
5. Verse. Rappaport and Kaplan. Urbach and Zach. Bernhardt, Zalewski and Burawski. Mook and Moore. Weidman (1933). Wile.
6. Gill. McGhee and Michelson. Berghausen. Jones.
7. Cabot. Fitchett and Weidman.

neally. The mice usually succumb to the infection within a month. If they do not, they should be killed, and cultures should be made from specimens obtained from the brain, liver, kidney, lung and spleen. Histologic sections will usually substantiate the cultural findings. Postmortem examination of the mice rarely reveals any gross pathologic change, despite extensive microscopic involvement, although occasionally cystic or nodular lesions the size of a pinhead can be seen.

In the tissues the organisms vary from minute, round objects, only a few microns in diameter, to large, round bodies, from 40 to 50 microns in diameter. The shape may be spherical or oval. The margin is rather sharp, and the larger cells frequently have a double contour. The formation of a capsule is not constant. The organism is variable in its reaction to stains. With hematoxylin and eosin stain they become slightly bluish or at times pink, or they may take no stain whatever, appearing as clear refractile bodies. With the Gram stain they show marked amphophilia, so that many of the organisms are intensely purple or even black while others take on a delicate shade of pink. Minute granules can often be seen within the bodies of these cells. In tissue, as in culture, reproduction is only by budding.

PATHOLOGIC PICTURE

According to the gross pathologic pictures the various forms of this disease have been classified by Freeman (1930 and 1931) into three types: (1) a purely meningeal form, with diffuse or granulomatous meningitis; (2) a perivascular form, with small granulomas or cysts in the cortex, extending from the surface of the brain along the course of the vessels into the cerebral parenchyma, and (3) an embolic form, with deeply placed lesions lying chiefly in the gray matter of the basal ganglions but occasionally in the white matter of both the cerebellum and the cerebrum.

TREATMENT

There is no specific treatment for this disease—all proved cases have been fatal. Some relief may be obtained by repeated spinal puncture to alleviate the symptoms due to the increased intracranial pressure.

COMMENT

The first case reported was unusual in that the duration of the disease was prolonged. An illness of five and one-half years is the longest on record (the longest duration previously reported [Urbach and Zach] was two and one-half years). Despite this prolonged period of infection the cerebral invasion was well localized to the meninges and choroid plexus, and general invasion did not take place by way of the ventricular system or the meninges. The early finding of "300

cells in the spinal fluid, with many small monocytes of various sizes and staining depth," strongly suggests that Torula was present then but not recognized, despite cultures and guinea-pig inoculation.

In many other cases reported the specimens of cerebrospinal fluid which were sent for culture were said to be sterile. This may have been due to the following facts: First, the laboratory technician may not have been familiar with this organism. Second, only minute amounts of the fluid may have been used on or in the culture mediums. Third, even under optimum conditions it may be four days at the earliest before a small colony the size of a pinpoint makes its appearance. Most cultures made as a routine will have been discarded by then and reported as sterile or showing no growth. Fourth, only ordinary culture mediums may have been used. In cases of suspected torulosis sugar containing mediums which favor a more rapid growth likewise should be employed. Into the tube of Sabouraud's agar slant or broth 2 cc. of spinal fluid may be introduced and all inoculations may be made directly from the puncture needle, so as to insure the injection of a sufficient amount of material and to prevent the possibility of contamination. This will also prevent attributing the yeastlike colonies which may be obtained on cultivation to contaminants.

SUMMARY

Torula infection of the central nervous system is a chronic disease with clinical manifestations resembling those of tuberculous meningitis, tumor or abscess of the brain or encephalitis caused by a yeastlike budding organism, Torula. This organism is widely distributed in nature and is ordinarily nonpathogenic. It is distinguished from other similar organisms by the absence of mycelia and spore formation, the presence of a capsule and little tendency to ferment sugars. Certain strains, however, have pathogenic properties and manifest a special predilection for the central nervous system.

The disease sets in insidiously with persistent severe headache, stiffness of the neck and vomiting, and later dimness of vision or actual blindness, paralysis and convulsions may occur. As a rule the patient is afebrile, and the symptoms of an acute infection are absent. Stiffness of the neck, neuroretinitis or choked disks, diplopia, nystagmus, strabismus and variable changes in the reflexes are the outstanding physical findings. The blood count and blood chemistry reveal no characteristic picture. The spinal fluid is under increased pressure and is clear or xanthochromic, with an increased number of cells, chiefly lymphocytes, and an increase in the amount of albumin and of globulin. The colloidal gold curve is most often of a meningitic type. The diagnosis can be made ante mortem only by the identification of the causative organism in the spinal fluid. There is no effective treatment, the course

of the disease being steadily downward, with a fatal termination. Pathologically there is noted granulomatous meningitis; and if the cerebral tissue is involved, cystic and granulomatous lesions are present, with relatively slight inflammatory reaction.

Torulosis of the central nervous system is relatively easy to recognize, if it is kept in mind and if a diligent search is made in the spinal fluid for the causative organism. It should be considered as a possibility in cases of obscure cerebrospinal disease, especially those characterized by severe headaches without a definite etiology.

Dr. Harold N. Cole gave many helpful suggestions during the preparation of this paper, and my colleagues at the Cleveland City Hospital assisted in various ways. Dr. G. Y. Rusk, of the department of pathology, the Mount Zion Hospital, San Francisco, made the photomicrographs and reviewed the histologic sections. Dr. Fred Weidman, of the department of dermatologic research, the University of Pennsylvania Medical School, permitted me to make a study in his laboratory of the various strains of Torula.

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UNUSUAL GLYCOGEN STORAGE IN A CASE OF DIABETES MELLITUS

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In fatal cases of diabetic acidosis the stores of glycogen in the body are, as a rule, markedly depleted. The importance of the case to be described lies in the paradoxical presence of an enlarged liver containing an excessive amount of glycogen in a diabetic patient who died in acidosis.

REPORT OF CASE

Clinical History.—M. R., a white man aged 24, was admitted to Duke Hospital on Nov. 10, 1934, in severe diabetic acidosis. There was no family history of diabetes.

In February 1930, while at work in a college laboratory, he found that his urine gave a positive reaction for sugar. This was not confirmed by the physician of the college. In September 1930 the patient began to lose weight and to have polyuria, polyphagia and polydipsia. Two months later, while he was in the college infirmary for an unrelated cause, glycosuria was again detected. His family physician prescribed a diet with 15 units of insulin three times daily. The patient did not follow this regimen but soon fell into the habit of taking 20 or 30 units of insulin per day as he saw fit, covering each dose with a glass of orange juice.

On Feb. 6, 1931, he was admitted to Duke Hospital in moderate acidosis. There were signs of a considerable loss of weight and dehydration. He had an infection of the upper respiratory tract, and there was a furuncle over the right eye. No other abnormal physical findings were noted. The edge of the liver could not be palpated. It was recorded that the hepatic dulness extended for 11 cm. above the right costal margin. At the time of the patient's admission to the hospital the sugar content of the blood was 480 mg. per hundred cubic centimeters, carbon dioxide-combining power was 44.7 volumes per cent and the cholesterol content was 214 mg. Some difficulty was experienced in regulating the diabetes. However, after thirteen days his condition was considerably improved, and he was discharged on a diet of carbohydrate 100 Gm., protein 100 Gm. and fat 200 Gm., with 30, 30 and 20 units of insulin per day.

In September 1933 the patient returned to the outpatient clinic, and urinalysis revealed a 4 plus reaction for sugar and a 2 plus reaction for diacetic acid. He admitted having difficulty in following his diet, which was then changed to carbohydrate 140 Gm., protein 70 Gm. and fat 150 Gm. per day.

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He was not heard from again until his final admission to the hospital. He had not kept to his diet, and for two or three months he had felt that the diabetes was becoming worse. He was losing weight and taking larger doses of insulin. On the rare occasions when he examined his urine it invariably gave a 4 plus reaction for sugar.

Six days before his final admission to the hospital he had what appeared to be a digestive upset, with nausea, vomiting and abdominal cramps. In spite of weakness, dyspnea and fatigue, he continued to work, and he was finally brought to the hospital in profound acidosis, with circulatory collapse bordering on a state of shock. He was in severe respiratory distress and was markedly dehydrated and stuporous. The edge of the liver was palpable 8 cm. below the right costal margin. No other significant physical findings were noted.

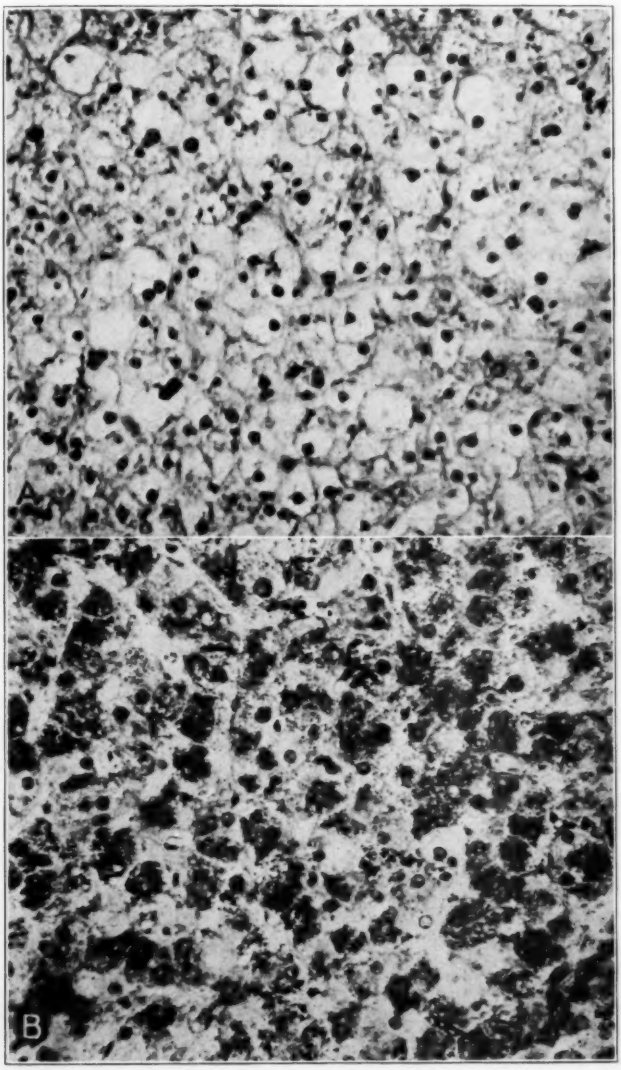
The urine contained 5 per cent sugar and large amounts of ketones. The carbon dioxide-combining power of the plasma was 17.4 volumes per cent. The sugar content of the blood ten hours after the patient's admission to the hospital was 320 mg. per hundred cubic centimeters. In spite of the administration of insulin and caffeine and of fluids and dextrose intravenously, the patient died twelve hours after entry.

Gross Postmortem Examination (Nine Hours After Death).—The liver extended 9 cm. below the right costal margin and weighed 2,700 Gm. The surface was smooth. The cut surface was waxy yellow and swollen. Each of the kidneys weighed 220 Gm. and had a smooth surface. On the cut surface pronounced yellow striations were noted in the region of Henle's loop. The heart weighed 250 Gm. and showed no hypertrophy or dilatation. The valves appeared normal. The pancreas weighed 100 Gm. and showed no gross abnormality. The adrenal and thyroid glands showed normal size and consistency. Permission to open the cranial cavity was not granted.

Microscopic Postmortem Examination.—The hepatic cells were greatly enlarged, and the cell membranes were fairly well outlined with eosin (fig. *A*). The cytoplasm was packed with fine granules. Some of the nuclei were enlarged and had a ringlike appearance. A few of the hepatic cells immediately surrounding the central veins were vacuolated. There was no congestion, cirrhosis or hemochromatosis. In the kidneys the epithelial cells of the loops of Henle were large, pale and vacuolated. There was also slight vacuolation of the epithelium of a few convoluted and collecting tubules. There was no vacuolation of the muscle fibers of the heart or voluntary muscle. The islands of Langerhans and the adrenal and thyroid glands appeared normal.

Pieces of liver, kidney and myocardium, after being left in a dilute solution of formaldehyde U. S. P. (1:10) for two months were dehydrated with absolute alcohol, mounted in pyroxylin and stained with Best's carmine for glycogen. The great distention of the hepatic cells was due to the presence of large amounts of glycogen within the cytoplasm (fig. *B*). The cells nearest the central veins contained somewhat less glycogen than those nearer the portal areas. There was also some glycogen in the ringlike nuclei. The vacuolated epithelium in the loops of Henle of the kidney contained large amounts of glycogen, and the epithelium of the convoluted and collecting tubules contained smaller amounts. The myocardium contained no glycogen. After the tissues had been preserved in a modified Kaiserling no. 3 solution[1] for eighteen months, more sections were taken, dehydrated

1. An aqueous solution of sodium acetate, 1 per cent; glycerin, 2 per cent, and a dilute solution of formaldehyde U. S. P. (1:100).



A, photomicrograph of a section of the liver. The granules of glycogen in the cytoplasm have been dissolved out, giving the cells a vacuolated appearance. Hematoxylin and eosin stain. *B*, hepatic cells packed with many fine granules of glycogen. Best's carmine stain.

with absolute alcohol and mounted and stained for glycogen as before. The liver and kidneys still contained large amounts of glycogen, although the quantity appeared reduced.

Frozen sections of heart, liver and kidneys from formaldehyde-fixed tissues were stained with scarlet red and hematoxylin. The hepatic cells immediately surrounding the central veins contained small amounts of fat. The heart and kidneys contained no fat.

Anatomic Diagnosis.—The diagnosis was hepatomegaly and abnormal storage of glycogen in the liver and kidneys (history of severe diabetes mellitus and death in acidosis).

Chemical Studies.—When examined two months after autopsy the preserving fluid appeared milky. A qualitative test for glycogen by the iodine method was strongly positive. On the addition of 95 per cent alcohol to the fluid a white precipitate resulted. After acid hydrolysis of the precipitate a positive reaction to the Benedict test was obtained. With phenylhydrazine, typical dextrosazone crystals were formed.

After eighteen months in the Kaiserling solution portions from different areas of the liver were taken for the determination of the glycogen content by the method of Good, Kramer and Somogyi.[2] The average of six determinations was 3.18 per cent, with a variation between 3.09 and 3.56 per cent. The fluid content varied between 71 and 72 per cent, averaging 71.6 per cent. Hence, the average glycogen content per hundred grams of dry liver was 11.2 Gm. The glycogen content of the kidney was 0.22 per cent for wet tissue, or 0.76 per cent for dry tissue.

COMMENT

The usual postmortem observations on patients with untreated diabetic coma include an increased deposition of glycogen in the nuclei of the hepatic cells, in the heart muscle and in the convoluted tubules of the kidney, with depletion of glycogen in the skeletal muscle, skin and cytoplasm of the hepatic cells.[3]

Our patient had severe diabetes and died in acidosis, yet large amounts of glycogen were demonstrated in the cytoplasm of the hepatic cells and the liver was enlarged. Moderate amounts were present in the nuclei of the hepatic cells and in the tubular epithelium of the kidneys; none was present in the skeletal muscle or myocardium.

The increase in the size of the liver in diabetes has been ascribed to infiltration of fat, cirrhosis and chronic passive congestion. In our patient the enlargement must have been due to a massive deposition of glycogen in the hepatic cells. Unfortunately, the available data are insufficient to enable us definitely to explain the presence of the excessive amount of glycogen in the liver, although two lines of reasoning are open.

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2. Good, C. A.; Kramer, H., and Somogyi, M.: The Determination of Glycogen, J. Biol. Chem. **100**:485, 1933.

3. Warren, Shields: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1930.

There is the possibility that our patient had glycogen storage disease (von Gierke [4]) and survived to young adulthood, when diabetes developed. Von Gierke's disease has been described as occurring in infancy and childhood and is characterized by early enlargement of the abdomen, due to storage of excessive amounts of glycogen, with marked hepatomegaly. The glycogen is remarkably stable, as shown by its slow response to epinephrine and in the delayed postmortem glycogenolysis.[5] The heart and kidney, too, may be the sites of storage of glycogen.[6] Also characteristic are hypoglycemia with ketosis during fasting, a flat, prolonged dextrose tolerance curve and unusual sensitivity to insulin. Tests of this nature had, of course, never been made in this case.

Parnas and Wagner [7] reported a case which, in the light of our present knowledge, was probably one of glycogen storage disease in which diabetes developed. Gjuric [8] recently reported two cases in females that fall into this category, one patient being aged 12 and the other 25. These patients at the time of the report required only small doses of insulin.

In our case there was no childhood history of abdominal enlargement, and hepatomegaly was not noted at the time of the patient's first admission to the hospital. It is possible that hepatomegaly was missed, but this is rather unlikely, since the examiner recorded even the upper margin of hepatic dulness.

The presence of glycogen in the tubular epithelium of the kidney is of no assistance in the diagnosis of glycogen storage disease, because it is frequently present also in diabetes mellitus.

The second line of reasoning has to do with the effects of insulin on the deposition of glycogen in the liver. That the glycogen content can be raised to normal in the liver of a diabetic patient by the use of insulin is generally agreed,[9] but that excessive amounts can so be

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4. von Gierke, E.: Hepato-Nephromegalia glykogenica, Beitr. z. path. Anat. u. z. allg. Path. **82**:497, 1929.

5. van Creveld, S.: Investigations on Glycogen Disease, Arch. Dis. Childhood **9**:9, 1934.

6. Humphreys, E. A., and Kato, Katsuji: Glycogen-Storage Disease, Am. J. Path. **10**:589, 1934.

7. Wagner, R., and Parnas, J. K.: Ueber eine eigenartige Störung des Kohlenhydratstoffwechsels und ihre Beziehungen zum Diabetes mellitus, Ztschr. f. d. ges. exper. Med. **25**:361, 1921. Parnas, J. K., and Wagner, R.: Beobachtungen über Zuckerneubildung, Biochem. Ztschr. **127**:55, 1922.

8. Gjuric, A.: Glycogen Storage Disease in Diabetes Mellitus, Časop. lék. česk. **74**:514 (May 10) 1935.

9. Joslin, E. P.: The Treatment of Diabetes Mellitus, ed. 5, Philadelphia, Lea & Febiger, 1935. Warren.[3]

stored has not been proved. Wilder, Allan, Power and Robertson [10] reported that the 3,300 Gm. liver of a patient with hyperinsulinism due to carcinoma of the islands of Langerhans contained 8.25 per cent glycogen. These observations indicate that the liver of a nondiabetic subject may store glycogen in excess under the influence of large amounts of insulin, here produced by the tumor and its metastases. In our case the history suggested that the amount of insulin taken, together with a diet high in carbohydrate, might have resulted in changes similar to those noted by Wilder and his associates. A survey of the other cases of spontaneous hypoglycemia that have been reported has revealed no record of enlargement of the liver. Hanssen [11] noted enlargement of the liver in a few young patients who had diabetes that was difficult to control and who had received large amounts of insulin. In these patients the size of the liver returned to normal after regulation with a diet lower in carbohydrate. Infiltration of the liver with fat was assumed to have caused the enlargement, but no biopsy data were given to confirm that point.

In our case the enlargement of the liver and the persistence of 3.18 per cent glycogen in the hepatic tissue after eighteen months in an aqueous preserving fluid (which had extracted a considerable quantity) suggest that an exceedingly large amount of glycogen must have been present at the time of death or that some type of hepatic fixation of glycogen was present. Unfortunately, the special clinical and postmortem tests that might have confirmed or disproved the latter possibility were not carried out.

SUMMARY

Hepatomegaly associated with the storage of a large amount of glycogen was noted in a diabetic patient who died in acidosis. Two possible explanations for this unusual condition are considered: first, this was a case of glycogen storage disease (von Gierke) in a subject who had survived to young adulthood, when diabetes developed, and, second, the glycogen was stored in the liver under the influence of large doses of insulin plus a high intake of carbohydrate.

10. Wilder, R. M.; Allan, F. N.; Power, M. H.; and Robertson, H. E.: Carcinoma of the Islands of the Pancreas, J. A. M. A. **89**:348 (July 30) 1927.

11. Hanssen, P.: Enlargement of the Liver in Diabetes Mellitus, J. A. M. A. **106**:914 (March 14) 1936.

CHLOROMA

REVIEW OF THE LITERATURE FROM 1926 TO 1936 AND REPORT OF THREE CASES

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CHICAGO

A chloroma is any tumor which has a green color in the fresh state.[1] Lehndorff in 1910 tabulated the data for the seventy-three cases of chloroma which had been reported up to that time. In 1926 Brannan summarized seventy-four reports of cases which appeared from 1910 to 1926, of which fifty-six were definitely cases of chloroma, the others being dubious examples. This present report brings the literature of chloroma up to date. The "Quarterly Cumulative Index Medicus" lists sixty-six references to papers, purporting to describe about eighty cases. Thirteen of these articles were not available for study, and twelve of the indexed papers did not concern chloroma. Of the fifty-eight cases summarized in table 1 (including three reported here for the first time), forty-six were unquestionable, while in ten cases there was a complete clinical syndrome of cranio-orbital tumor with leukemia, but no autopsy or biopsy was made in which the color of the tumor was noted. In the other two cases there was the classic syndrome, with greenish tissue visible ante mortem, but no necropsy was made. To be strictly academic, then, the present tabulation adds forty-six cases to the one hundred and twenty nine already reported.

Lehndorff's study showed that in fifty-six cases the condition was considered to be of lymphoid origin and in seventeen of myeloid origin. Brannan stated that in forty-nine of the cases which he studied the origin was definitely myeloid. Burgess stated as his conviction that in all cases of chloroma the condition is of myeloid origin. Askanazy offered a similar view. Current textbooks vary as to the opinion given: MacCallum, Naegeli, Aschoff and Ewing have described both myeloid and lymphoid forms. Mallory, Karsner, Wells, Piney and Boyd have stated that the process is always myeloid; although Boyd said he was not convinced that the myeloblast is the responsible cell. In fifty of the cases considered in this paper the condition was said to be of myeloid origin, and in only five of the indubitable examples was it stated to be of lymphoid origin. The basis for this statement was always that the large mononuclear cells in the blood and in the tumors were called

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TABLE 1.—*Summary of Cases of Chloroma* *

Author	Age	Sex	Site of Lesion: Skeleton	Lymph Nodes	Spleen	Nervous System	Post-mortem Study	Leukocytes	Immature Cells: Type	Per Cent
Unquestionable Cases										
Müller	9 yr.	M	p	p	p	o	p	120,000	Myeloblasts	72.0
Rothschild	11 yr.	M	o	o	o	p	p	6,000	Normal blood	
Blatt	3 yr.	F	p	p	o	p	o	13,000	Myeloblasts	4.5
Clivio and Mascherpa	9 yr.	M	p	o	o	p	p	6,000	Large mononuclears	48.0
Feer, case 4	4 mo.	M	p	p	p	o	p	104,000	Lymphoblasts	89.0
Case 6	5 yr.	M	p	p	p	o	p	34,300	Myeloblasts	97.0
Reitano	48 yr.	M	p	o	o	p	p	1,160-38,000	"Blastic"	
Haintz	23 yr.	M	p	o	p	p	p	14,500	Myeloblasts	30.0
Rosenblum and Lebedewa	14 yr.	M	p	p	p	o	p	11,300	Myeloblasts	66.0
	18 yr.	M	p	p	p	o	p	5,400	Myeloblasts	67.0
Seemann and Sajzewa	42 yr.	M	p	p	p	o	p	45,000	Myeloblasts	5.0
									Eosinophils	49.0
Monger	16 yr.	M	p	p	p	p	p	16,000	"Blasts"	30.0
Critchley and Greenfield	19 yr.	M	p	p	p	p	p	229,000	Myeloblasts	50.0
	8 yr.	M	p	p	o	p	p	15,700	Myeloblasts	3.0
Weil, Isch-Wall and Bertrand	27 yr.	F	p	o	p	..	..	97,200	Undifferentiated	33.0
Reddick and Brandes	28 yr.	M	o	o	o	..	p	18,700	Myeloblasts	57.0
de Asua	17 yr.	F	o	o	p	o	p	49,000	Many "blasts"	
Zeiss	12 yr.	F	p	p	..	p	p	20,800	Lymphoblasts	26.0
Gamble	7 yr.	F	p	p	o	p	o	34,000	Myeloblasts	20.0
Bamforth and Edwards	19 yr.	F	p	p	o	o	p	8,300	Normal blood	
	39 yr.	F	p	p	..	p	p	25,600	Primitive lymphocytes	20.0
	33 yr.	M	p	o	o	o	p	34,000	Lymphoblasts	69.0
	4½ yr.	M	o	p	p	..	p	132,000	Primitive cells	79.0
Ashby	9 yr.	M	p	p	p	..	p	22,000	Myeloblasts	71.0
Ashby and Smith	2½ yr.	M	p	o	o	p	p	14,200	Myeloblasts	59.0
Washburn	22 mo.	M	p	o	o	o	o	18,000	Myeloblasts	2.0
Swanson	2 yr.	F	p	p	p	..	..	76,000	Myelocytes	0.5
Allen	4½ yr.	F	p	p	p	p	o	41,000	Myeloblasts	29.0
Derman and Lifschitz	30 yr.	F	o	p	p	o	p	15,000	Myeloblasts	31.0
	11 yr.	M	o	p	p	o	p	7,800	Monocytes	58.0
								5,000	Myeloblasts	68.0

Quincke	37 yr.	M	p	o	p	..	..	3,000	Lymphoblasts	30.0
Hertz	11 yr.	F	p	p	o	o	o	15,000	Large lymphocytes	20.0
Doub and Hartmann	17 yr.	M	p	p	p	o	o	24,500	Myeloblasts	83.0
Strasser	58 yr.	F	o	o	o	o	p	1,100-310	Myeloblasts	4.0
Cohen (1924)	4½ yr.	M	p	o	o	o	o	3,600	Myeloblasts	4.0
Kandel	22 yr.	F	o	o	o	o	p	1,200	Myeloblasts	40.0
	25 yr.	M	o	p	p	o	p	299,000	Myeloblasts	20.0
	13 yr.	F	o	p	p	p	p	36,100	Myeloblasts	5.0
Lecène	23 yr.	M	p	o	o	o	o	.....	Normal blood	
Heiberg	2 yr.	M	..	..	..	..	..	.....	.....	....
	5 mo.	M	..	..	..	..	..	.....	.....	....
Krumbein	3 yr.	M	o	p	o	p	p	.....	.....	....
	6 yr.	M	..	..	..	..	..	.....	.....	....
	14 yr.	F	..	..	..	..	..	.....	.....	....
Castorina	5 yr.	F	p	p	p	p	o	8,200	Metamyelocytes	4.0
Nasso	6 yr.	M	p	p	p	o	p	4,680	Myelocytes	15.0
			Clinical Syndrome; Green Tissue Visible							
Levy and Laff	3 yr.	M	p	o	o	o	o	60,400	Myelocytes	92.0
Perazzi	3½ yr.	M	p	p	p	..	o	14,000	Metamyelocytes	2.0
			Clinical Syndrome; No Biopsy							
Volpe	22 mo.	M	p	p	p	o	o	11,000	Myeloblasts	28.0
Ashby and Smith	4 yr.	M	p	o	o	..	o	27,600	Myeloblasts	2.4
Nasso	7 yr.	M	p	p	p	o	o	17,500	Myeloblasts	5.0
	7 yr.	M	p	o	p	o	o	6,250	Myelocytes	10.0
	9 yr.	M	p	o	..	o	o	5,630	Myelocytes	4.0
Castana	15 mo.	M	p	p	p	o	o	14,000	Myeloblasts	1.0
Castorina	10 mo.	F	p	p	p	o	o	16,600	Myeloblasts	4.5
	2 yr.	M	p	o	p	p	o	20,000	Monoblasts	10.0
Gerbasi	20 mo.	F	p	o	p	o	o	17,160	Immature cells	5.0
Gandolfo	28 mo.	F	p	p	p	o	o	21,800	Myeloblasts	4.5
									Hemocytoblasts	42.0

* p indicates present during life, and o indicates absent during life.

lymphocytes or lymphoblasts and the reaction of the tissues for peroxidase (if studied) was negative.

The clinical features of the cases reported were as protean as the histologic material was specific. The green tumor was observed invading all parts of the body, and the variety of the involvement was responsible for the diverse clinical symptoms. Two main clinical types occurred, however: One was the so-called classic syndrome, in which a rapidly growing orbital tumor in a child resulted in profound proptosis. Associated with this were varying degrees of cranial tumefaction, lymphadenopathy and grossly noticeable involvement of other bones. Rapidly increasing, intractable anemia was frequently noted, and many of the papers recorded erythrocyte counts of less than 1,500,000. Splenic enlargement was not frequent, and a hemorrhagic diathesis was rare. The other type tended to occur in older patients and consisted of a rapid

TABLE 2.—*Summary of Data*

	Number of Cases
Males	38
Females	20
Over 18 years old	15
Less than 18 years old	43
Leukocytes less than 6,000	8
6,000 to 15,000	13
Over 15,000	31
Leukemic blood	50
Normal blood	3
Diagnosis of myeloid leukemia	50
Diagnosis of lymphoid leukemia	6

decline in the patient's strength, profound anemia, varying amounts of osseous pain and gross bony enlargement and usually enlargement of the lymph nodes and spleen. Eight patients had leukocyte counts of less than 6,000 and three (Strasser; Reitano; Kandel, case 1) showed leukopenia (less than 2,000 leukocytes). In these cases necrotizing angina, stomatitis with bleeding and cerebral hemorrhage, respectively, occurred.

The common clinical feature in all these cases was the leukemia, with the exception of three cases (Rothschild; Bamforth and Edwards, case 1; Lecène) in which the leukocyte count was reported as normal. The leukocyte count varied from 299,000 (Kandel, case 2) to 310 (Strasser); and in the majority of cases a diagnosis of myeloid leukemia was made. In general, the percentage of myeloblasts was about 50, with extremes of 0 and 97 per cent (Feer, case 4). One patient (Levy and Laff) had 92 per cent myelocytes and no myeloblasts; another was unique in displaying 49 per cent eosinophils (Seemann and Sajzewa) in the peripheral blood. Frequently the differential count was described as consisting of primitive cells, undifferentiated cells, hematogonium

and hemohistioblasts (Gandolfo). On the stained films the anemia was apparent, and many nucleated and young erythrocytes were seen. Lecène's case was unusual: the patient had a tumor of the humerus, which when excised was said to be a myelocytic chloroma. The blood was normal. The patient was given roentgen therapy, and he was living and well two years later. Only one other patient survived for as long as that, Washburn's patient—a 22½ month old boy. A green tumor diagnosed as a myelocytoma was excised from the skull, and another tumor in the femur was treated with roentgen rays. The blood is reported as having been questionably leukemic before therapy was started and as becoming normal afterward. This boy was living and well two and one-half years later. The remainder of the patients died rather promptly, few surviving for eight months after the symptoms appeared.

Many different terms were employed in describing these cases, but the majority of authors (the best usage) called the classic syndrome in children chloroma. The older patients with classic myeloid leukemia clinically and green tumors at necropsy were designated as having chloroleukemia. The leukemia was usually called acute myeloid or myeloblastic; although in ordinary usage the term acute is reserved for conditions in which the blood contains in excess of 90 per cent myeloblasts and which are of short duration. Certainly, the brief duration of life in most of these cases of myeloid leukemia justifies a diagnosis of acute myeloid leukemia.

Other laboratory studies were not relevant, except that only one author (Gandolfo) reported the presence of Bence-Jones' proteose in the urine, although many reported its absence. The calcium and phosphorus contents of the serum were generally normal, and the phosphatase content was never determined. Treatment was varied but was mostly supportive and symptomatic. Several of the German writers reported injecting neoarsphenamine to no avail. If roentgen therapy was used, the patient's condition generally became worse. This is just what happens in acute myeloblastic leukemia, and the bad result in chloroma helps justify calling the leukemia acute in the absence of the usual hematologic criterion. One patient (Kandel, case 2) was given roentgen therapy and died a month later, after a rapid decline. The same happened in several other cases and was especially striking in one case (Doub and Hartmann), in which the leukocyte count fell from 24,500 (83 per cent myeloblasts) to 1,150 in two days.

The roentgenologic examination yields variable results. In young children the only finding may be a separation of the cranial sutures (Cohen, 1924). Periosteal elevation is probably the most common finding and may give either of two appearances: It may be lamellated

and resemble scurvy or, if ossification is occurring, Ewing's tumor; or there may be radiating spicules of ossifying material perpendicular to the shaft, in which case it resembles osteogenic sarcoma. There may be simply a generalized osteoporosis, or there may be multiple punched-out, fuzzy or sharp osteolytic lesions (Bamforth and Edwards; Doub and Hartmann), usually in the diaphysis and in the skull, and then the resemblance to multiple myeloma or metastatic carcinoma is striking. Massive rarefactions have been described (Washburn). Allison, and Doub and Hartmann have given good descriptions of the roentgenographic picture. The rather high incidence of bony involvement, when looked for, is in striking contrast to the situation in myeloid leukemia, in which Craver reported but one example of an osseous lesion in a series of eighty-two patients. The same paper reports a 7 per cent incidence of osseous lesions in lymphoid leukemia. The lesions in either type are indistinguishable from those reported in chloroma, a fact emphasized by Clark.

The differential diagnosis varies with the clinical picture. In the classic cranio-orbital syndrome, without changes in the blood, other malignant growths of the orbit and cranium, Mikulicz' disease and osteomyelitis and abscess must be considered. Cohen (1934) reported a case of orbital lymphoma in a 65 year old man who had chronic lymphoid leukemia. The tumor was not green, and the course was slow. With roentgenographic evidence of bony involvement and no changes in the blood, the conditions referred to in the previous paragraph must be considered, as well as the essential xanthomatoses in which there is splenomegaly as well. The differentiation will eventually be histologic, multiple myeloma being composed of plasma cells and the other diseases showing specific lesions. If, however, a patient has myeloid leukemia and bony and orbital tumors the diagnosis of chloroma is probably correct.

Pathologically, when careful histologic studies are made with appropriate stains, the observations are specific. The tumors are either myeloblastomas or myelocytomas. Depending on which cell type predominates, the reaction for oxidase will be negative or positive (typical myeloblasts contain no specific granules to give the reaction for peroxidase). However almost any such tumor will have enough promyelocytes or myelocytes with typical granulations to make diagnosis possible with stains for peroxidase. These tumors are composed of large anaplastic-appearing cells, with little stroma, no reticulin and fair vascularity. There are seldom regions of erythroblastic activity, although biopsy of one tumor (Castana) was described as showing erythromyeloblastoma. Active invasion of surrounding structures is prominent. The tumor and infiltrations are directly continuous either with a subperiosteal

accumulation or with a metaplastic lymph node. This relation to bone is striking in the skull and in the thorax. In the skull, which is involved in 73 per cent of cases (Rothschild), the green mass is seen to be adherent to the sutures, growing over, under and into the dura, the paranasal sinuses and the orbit, and infiltrating the nerves, scalp, sclera, choroid (Cohen, 1924) and subcutaneous tissues. In the thorax there are almost always large sheets of tumor adherent to and growing from the under surface of the sternum, through the pleura, into muscles. In one case (Quincke) there were extension into the myocardium and almost complete stricture of the pulmonary artery with tumor tissue. Along the vertebral column a green tumor from the periosteum and the lymph nodes may invade the neural canal, nerves, erectores spinae muscles, fill the pelvis and infiltrate the ovaries. The bone marrow may or may not be green tinged and in general is not. It is usually hyperplastic, gelatinous and grayish and may be almost liquid. The histologic picture is typical of myeloid leukemia of an acute variety, with myeloblasts predominating. Lymph nodes show only infiltration, as does the spleen, the follicles in either cases showing no proliferation.

When the central nervous system is involved it is usually by pressure or by obliteration of the blood supply, with resulting necrosis. Plugs of myeloblasts and myelocytes have been described (Critchley and Greenfield) in the vessels of the cord, and perivascular cuffing when present is accomplished by similar cells. Discrete tumors in the neural tissues have not been described. The infundibulum and choroid plexuses of the ventricles were green and infiltrated in one of the cases described by Critchley and Greenfield, but extension into the white matter had not occurred. The invasion of the neural canal frequently produces transverse myelitis, and thrombosis of the arteries may cause extensive ischemic necrosis. Rothschild's patient showed signs of a focal cerebral lesion. When decompression was performed a green tumor was revealed. Krumbein's patient had mastoiditis and showed signs suggesting thrombosis of the lateral sinus and meningitis. Mastoidectomy disclosed green tumor in the cells, and autopsy revealed more in the cranial cavity. Sections frequently showed marked infiltration of the peripheral nerves, probably a potent source of the pain from which so many patients suffered.

The spleen and the liver rarely contain green tumor tissue, but the kidneys seldom fail to have it, diffusely or in nodules. The green tumor is generally located in the cortex. The pancreas usually escapes, as do the adrenal glands and the bladder. The ovaries are frequently grotesquely enlarged by the chloroma. Peyer's patches of the gastrointestinal tract are never reported as green. In all these organs the usual gray leukemic infiltration may be seen. The pathologic picture,

then, is that of true neoplasia in every respect, with the proliferating cells myeloblasts and myelocytes. The difference from ordinary leukemia is the tendency of the infiltrations to act as metastases do and spread locally and destructively. The type of invasion, except for its close relation to bones and its inevitable periosteal involvement, closely resembles grossly that seen in lymphosarcoma.

CASE 1.—A 22 year old white woman, a student, of Swedish ancestry, was first seen in the medical clinic of the University of Chicago on March 3, 1933. Her

TABLE 3.—*Blood Counts in Case 1*

Date, 1933	Hemoglobin, %	Erythrocytes	Leukocytes	Platelets
3/ 3	39	1,610,000	2,100	
3/20	32	1,630,000	3,400	176,000
3/21	28	1,580,000	1,000	160,000
3/22	30	1,400,000	1,600	120,000
3/23	36	1,580,000	1,280	
3/24	39	1,600,000	3,000	130,000
3/25	40	1,400,000	1,200	
3/27	30	1,480,000	1,280	108,000
3/28	32	1,520,000	1,800	
3/30	24	1,400,000	1,200	
3/31	24	.....	1,080	116,000
4/ 1	22	1,280,000	900	112,000
4/ 2	20	1,180,000	1,200	22,000

TABLE 4.—*Differential Leukocyte Counts in Case 1*

Date, 1933	Myelo-blasts	Pro-myelo-cytes	Myelo-cytes	Meta-myelo-cytes	Poly-morpho-nuclears	Lympho-cytes	Mono-cytes
3/ 3	39	1	..	..	12	42	6
3/20	38	2	..	3	12	45	..
3/21	30	4	..	..	8	58	..
3/22	40	2	1	6	..	50	1
3/23	30	3	1	1	10	55	..
3/24	25	4	2	5	14	50	..
3/25	20	6	3	4	10	57	..
3/27	39	4	7	5	..	45	..
3/28	35	2	2	2	8	50	1
3/30	30	2	..	..	12	54	2
3/31	34	6	5	..	20	30	5
4/ 1	26	6	4	2	27	27	2
4/ 2	22	10	7	8	17	34	3

complaints were of pallor and weakness for four months. Two months prior to her admission to the clinic she had been sick in bed for four weeks, with a sore throat, followed by pain in both legs and in the back and a low grade fever. In the month prior to her admission to the clinic she had been failing rapidly. The past history was otherwise noncontributory. The menses were normal, and the patient had never been pregnant. Physical examination showed marked pallor, no petechiae, a pulse rate of 100, a barely palpable liver and a small retinal hemorrhage. The blood pressure was 114 systolic and 70 diastolic. The blood counts are listed in tables 3 and 4. Hospitalization was advised but not accomplished until February 20, when the patient had become much sicker. On her admission to the hospital there were more retinal hemorrhages, and the pallor was more striking. The course in the hospital was rapidly down hill, with an increasing temperature, showers of petechial hemorrhages, vaginal bleeding, stomatitis and

amblyopia. Treatment was supportive, including blood transfusions. The patient died on April 3, approximately five months after the onset of the illness.

Gross Postmortem Examination (by Dr. P. R. Cannon).—The liver was of normal size, weighing 1,200 Gm. It was flabby, and the capsular surfaces were smooth. Cut surfaces were brown, with indistinct lobular markings. There was no visible leukemic infiltration. The spleen was firm and weighed 160 Gm. It had a smooth purple capsular surface, with prominent follicles on the cut surface. The lymphoid follicles of the gastro-intestinal tract were not enlarged. The kidneys weighed 110 Gm. each and contained no gross leukemic infiltration. The ovaries were enlarged (8 by 7 by 4.5 cm.), hyperemic and hemorrhagic and contained numerous cysts. The cut surface showed that the organs were composed mainly of solid tissue the color of which was somewhat obscured by blood. The abdominal periaortic lymph nodes were diffusely enlarged and brownish green. These nodes extended up past the celiac axis. In the retroperitoneal tissues contiguous to the broad ligaments, particularly on the left, were large greenish gray nodes. The mesenteric lymph nodes were normal. The lungs were contracted and pinkish gray, with small patches of greenish tissue adherent to the visceral pleura of each apex. The heart was normal, as was also the skeletal musculature. The bodies of the third and fourth lumbar vertebrae contained a slightly greenish, gelatinous bone marrow, and similar marrow was seen in the ribs and sternum. Anteriorly, along each side of the vertebral column, greenish gray tissue was seen.

Histologic Examination.—The lymph nodes showed moderate hyperplasia of the reticular elements but not of the lymphoid follicles. The spleen showed hyperplasia of the red pulp, with many histiocytes enlarged and prominent. There was no conspicuous myelopoiesis, and the follicles were compact. The ovaries were almost completely replaced by a growth of neoplastic mononuclear cells with pale nuclei, prominent nucleoli and nongranular cytoplasm. There was no reticulin but much rhexis. Mitotic figures were frequent. These cells were identified as myeloblasts. The pelvic fat was infiltrated with these tumor cells, as were the nerves and skeletal muscle, in which there was also necrosis. The retroperitoneal (green) lymph nodes were completely replaced by tumor cells. There were no leukemic foci in the liver or kidneys. There was moderate hyperplasia of the lymphoid tissue in the intestines, and extension of the neoplastic tissue into the serosa was noted. The bone marrow was markedly hyperplastic, with many myeloblasts, which were readily identifiable by comparison as the tumor cells. The sections were characteristic of myeloid leukemia of an acute type.

Case 2.—A 25 year old white man, of American ancestry, was first seen in the medical clinic of the University of Chicago on Aug. 15, 1934. His complaints were of pain in the hips, swelling of the angles of the jaw and loss of 20 pounds (9 Kg.) within the past six weeks. His past and family histories were irrelevant. On examination pronounced pallor and cervical lymphadenopathy were obvious. The pulse rate was 108, the temperature 100 F. and the blood pressure 130 systolic and 70 diastolic. In the ocular fundi were hemorrhages and leukemic infiltration. The liver was palpable and smooth, 5 cm. below the margin of the ribs on the right. The spleen was palpable 7 cm. below the left ribs; neither the liver nor the spleen was tender. A few small axillary and inguinal nodes could be felt. Roentgenograms displayed small diffusely scattered infiltrative lesions in the apex of the left lung and subclavicular area. The patient entered the Billings Hospital on August 17 for roentgen therapy because of enlargement of the spleen and leukocytosis (see tables 5 and 6 for the results of the blood counts). In the next seven days he received a total of 1,185 roentgen units over the cervical nodes, spleen and right hip. He was discharged on August 27, feeling much better. He was

seen on September 6 in the hematologic clinic. He was feeling stronger and was given solution of potassium arsenite. On September 13 the temperature was 102 F. and the pulse rate 128, and the patient had a bad cold and cough. Rest in bed was ordered, but the cough became worse. He was readmitted to the hospital on September 19 in a moribund condition. He died the next day, approximately three months after the onset of symptoms.

Gross Postmortem Examination (by Dr. E. Humphreys).—There was a moderate amount of blood-tinged fluid in the pleural and pericardial cavities. Numerous petechial hemorrhages and suggillations were seen in the parietal pericardium and the epicardium. The heart was flabby, and the foramen ovale was

TABLE 5.—*Blood Counts in Case 2*

Date, 1934	Hemoglobin, %	Erythrocytes	Leukocytes	Platelets
8/16	51	2,580,000	276,000	200,000
8/17	48	2,980,000	248,000	
8/18	50	2,370,000	203,000	
8/19	46	2,040,000	121,000	
8/20	46	2,450,000	91,800	
8/21	48	2,430,000	115,000	
8/22	43	2,000,000	68,600	
8/23	52	2,650,000	79,000	
8/24	54	2,630,000	75,000	
8/25	50	2,410,000	47,800	
8/26	47	1,610,000	35,000	
8/27	51	2,450,000	31,000	50,000
8/28	49	.....	23,200	
9/ 6	48	2,710,000	43,000	
9/13	44	2,377,000	113,000	
9/19	28	1,470,000	157,400	
9/20	..	.....	299,000	

TABLE 6.—*Differential Leukocyte Counts in Case 2*

Date, 1934	Myelo-blasts	Pro-myelo-cytes	Myelo-cytes	Meta-myelo cytes	Poly morpho-nuclears	Lympho-cytes	Mono-cytes
8/16	12	6	4	12	45	8	13
8/20	7	8	4	16	52	2	11
9/ 6	8	6	4	15	43	9	15
9/13	9	16	12	20	29	8	7
9/19	20	13	4	9	38	5	11
9/20	13	11	14	27	25	3	7

patent. "Thrush breast" mottling of the endocardium and gray leukemic mottling of the myocardium were noted. The liver was large (2,250 Gm.) and firm, with no definite gross infiltration. The spleen was moderately firm and weighed 710 Gm. The cut surface was mottled brownish red. The gastro-intestinal tract displayed petechial hemorrhages and distinctly enlarged Peyer patches. The kidneys were large (290 and 220 Gm., respectively), and showed diffuse, nodular greenish infiltration of the cortex. This green tumor was estimated to replace fully 60 per cent of the cortex of one kidney. The lymph nodes were variable: the cervical nodes were not enlarged, while the tracheobronchial nodes were prominently enlarged and had a mottled gray-green cut surface, especially peripherally. The peripancreatic and periaortic and mesenteric nodes were enlarged and greenish. A blood clot from the portal vein was greenish red. The bodies of the lumbar vertebrae had marrow the color of which resembled that of the lymph nodes: greenish gray, with scarcely a tinge of pink. The marrow of the upper third of the femur was greenish gray, soft and abundant. The marrow of the ribs was scanty, semi-

liquid and greenish. When the periosteum was stripped from the sacrum, ribs and vertebral bodies a paper-thin layer of greenish tissue was seen loosely adherent to the bone.

Histologic Examination.—The bone marrow was a solid tissue with few fat cells and was made up chiefly of myeloid tissue. The predominant recognizable cells were of the leukoblastic series, and immature forms were more numerous than mature cells. In many regions the predominant cells were myeloblasts. In many regions the proportion of eosinophils was high, and most of the cells were myelocytes. One striking feature was the absence of typical megakaryocytes from the marrow. The spleen was packed with cells of the type seen in the marrow, with a few small hemorrhages and numerous hemosiderin-filled phagocytes. The liver contained a few small leukemic infiltrations about and in the triads. The lymph nodes showed varying degrees of leukemic infiltration and obliteration of the architecture. In the kidneys there were leukemic infiltrations in the cortex and medulla, hilar fat and calices. The myocardium contained many small leukemic infiltrations about the larger blood vessels. In the right atrium was a massive hemorrhage disrupting the muscle fibers, which were degenerating. The lungs had small confluent bronchopneumonia-like nodules composed mostly of immature cells. In the submucosa of the appendix was marked leukemic infiltration.

Case 3.—This patient was a girl aged 13 years who had been under the care of Dr. Marie Ortmayer, of Chicago. In June 1930 the abdomen began to enlarge and in October the cervical lymph nodes became large. The girl became sicker and weaker and was admitted to the Women's and Children's Hospital on March 23, 1931. The blood count at that time was as follows: hemoglobin, 55 per cent; erythrocytes, 2,410,000; leukocytes, 24,000; myeloblasts, 5 per cent; promyelocytes, 9 per cent; myelocytes, 13 per cent; metamyelocytes, 18 per cent; polymorphonuclears, 40 per cent; basophils, 2 per cent; lymphocytes, 9 per cent, and monocytes, 3 per cent. The patient rapidly became worse, and transverse myelitis, pericardial effusion, cardiac decompensation and trophic ulcers developed successively. She died in April 1931. The last blood count, made on April 4, showed: hemoglobin, 20 per cent; erythrocytes, 2,120,000, and leukocytes, 36,100. At autopsy green tumors were noted in the lymph nodes, ovaries, kidneys and bone marrow. The histologic diagnosis was acute myeloid leukemia. (This case will be reported in detail later by Dr. Ortmayer.)

These three cases are examples of the second type of chloromatous involvement, i. e., chloroleukemia. The diagnoses during life were, respectively, subacute myeloid leukemia with leukopenia, subacute myeloid leukemia and chronic myeloid leukemia. Green tumor tissue was found in all cases at autopsy, and histologically there was no question but that the process was a fairly acute myeloid leukemia. All the evidence gleaned from a study of the cases that have been reported favors the theory that chloroma is always associated with myeloid leukemia. In well studied cases of characteristic lymphosarcoma (Sternberg) and so-called leukosarcoma, which may be considered as lymphatic analogues of chloroma, green pigmentation has never been reported. Burgess said he felt that if the disease is ever of lymphoid origin, occasional cases in which there is a blood picture of chronic lymphoid leukemia (i. e., many small lymphocytes) should occur. This

is not unreasonable, for in the cases reported the blood picture varied all the way from profound neutropenia to that of the most acute myeloid leukemia. Cases have been reported in which the leukocyte count was as high as 1,880,000 (Burgess) and in which there were all the characteristics of chronic myeloid leukemia. Such a case as Burgess described has not been reported. In all the cases described as of lymphatic origin the blood contained large mononuclear cells, with nucleoli and irregular lobulated nuclei, called lymphoblasts, either because of their extrinsic appearance or because they did not show a reaction for peroxidase. It is admittedly difficult to differentiate isolated myeloblasts from lymphoblasts, and the stain for peroxidase is of no help, for neither type of cell has any granules (except the Auer bodies, which show a negative reaction for peroxidase). Only by clearly identifying less immature cells as of one series or another can this reaction be of value. The true distinction should be made from histologic preparations of the marrow and lymph nodes with appropriate stains for granules, such as the Maximow stain. In the cases described in which the condition was of lymphoid origin the description of the marrow and nodes was not specific and could not be considered as convincing.

The green pigment is a source of great interest. It is apparently a lipochrome and is said to contain iron. The one certain feature is that it disappears in about an hour but may be recalled with hydrogen peroxide. It is important to remember that no lymphoblastoma is ever green pigmented. The myeloid neoplasm of the common fowl (Mathews) called, curiously, leukochloroma, is not green and is composed solely of neoplastic myelocytes. On the other hand, green myelocytomas are reported in man. The pigment is ordinarily attributed to the myeloblasts and the eosinophils. The tumors of the patient with eosinophilic leukemia (Seemann and Sajzewa) were no greener than those of any other patient. In the three cases reported in detail in this paper no study was made of the pigment.

Discussions as to the significance of chloroma are plentiful in the literature. It is generally asserted that chloromas "prove" or suggest strongly the close relation, if not the identity, of myelogenous leukemia and neoplasms in general. The invasive feature of the chloroma is the point emphasized for the argument. It has never been apparent why the fact that myeloid cells in classic cases of leukemia do not erode bone is considered inferentially as evidence against the presence of a neoplasia. But such may be the case, for this fact is seized on in discussions of chloroma to demonstrate that myeloid cells (which are concomitantly causing leukemia and are capable of infiltrating the skin, heart and liver and of producing metaplasia in the lymph nodes and

spleen) are neoplastic. It should be apparent that the myeloid cells, specifically the multipotential myeloblasts, in chloroma simply assume another rôle and become capable of destructive invasion and of forming or cooperating in the forming of green tumors.

SUMMARY AND CONCLUSIONS

The literature on chloroma is reviewed from 1926 to the present, and three new cases are reported.

One hundred and seventy-five indubitable cases of chloroma have been reported in the world literature.

With recent improvements in staining technic and better differentiation of the acute lymphoid and myeloid leukemias, almost all the recent cases of chloroma have been reported as cases of myeloid leukemia, several standard texts to the contrary.

The inevitable association of chloroma with myeloid leukemia should make it obvious that chloroma is simply a variant of myeloid leukemia, with the multipotential myeloblast assuming the distinctive rôle as type cell of an invasive neoplasm.

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TREATMENT OF OCCLUSIVE ARTERIAL DISEASE OF THE EXTREMITIES BY PASSIVE VASCULAR EXERCISE

REPORT OF SIXTY-EIGHT CASES

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The old idea that the minute volume of blood flowing through distal parts of the body can be increased by lowering the pressure of the air in contact with these parts, which was first utilized therapeutically over a hundred years ago, has been revived again recently and, chiefly through the efforts of Louis G. Herrmann, has been applied on a larger scale than ever before in the treatment of occlusive arterial disease of the extremities. The history of this method, illuminated by voluminous quotations and an exhaustive bibliography, the theory on which it is based and the modern technic of its application are given in Herrmann's [1] monograph, but many more data bearing on the results of its employment must be tabulated before its value and applicability can be ascertained exactly. It is to help satisfy that desideratum that this report [2] is presented.

METHOD

We planned to treat each affected extremity for one hundred hours, at the rate of five (consecutive) hours daily, by alternately raising and lowering the environmental pressure 20 and 80 mm. of mercury, respectively, four times a minute, but it was often necessary to modify this program to comply with various exigencies. In some cases satisfactory results were obtained in less than one hundred hours, whereas in others it seemed advisable to continue the treatment much longer. The five hours per diem were not always consecutive hours. Occasionally treatment was omitted entirely for a day or two, and the individual periods of treatment were not infrequently short and irregularly spaced. In a few cases 2 ounces (60 cc.) of whisky was given just before each period of treatment, but other forms of adjuvant treatment, such as local artificial hyperthermia, active vascular exercises and vasodilator drugs, were dispensed with as far as possible

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1. Herrmann, Louis G.: Passive Vascular Exercises, and the Conservative Management of Obliterative Arterial Diseases of the Extremities, Philadelphia, J. B. Lippincott Company, 1936.

2. A preliminary report has appeared (J. Iowa M. Soc. **25**:549, 1935).

in order to allow an unobstructed view of the results of varying the environmental pressure. Ulcers were left undisturbed, except for a protective covering. Much difficulty was experienced with the crude rubber cuffs which were originally furnished with the pavaex boot. They were often uncomfortable; frequently they were so tight or so loose that effective treatment was impossible, and in many instances they were difficult to apply and remove.

In two years sixty-eight patients received a total of five thousand, nine hundred and forty-seven hours of treatment. There were thirty-nine cases of arteriosclerosis obliterans, eight of thrombo-angiitis obliterans, twelve of sudden occlusion of a major artery and nine of miscellaneous disorders. The selection of cases was made purely on clinical grounds, without the employment of a special diagnostic procedure of any kind. Patients were accepted or rejected strictly on the merits in each case, but no patient was refused treatment simply because a good result seemed unlikely.

RESULTS

At the end of thirty hours of treatment it was often possible to predict what the final result would be. Rest pain was usually relieved during actual treatment, but frequently it recurred in the intervals between treatments and in a few cases seemed to be aggravated by the treatment. Cyanosis was lessened, as a rule. Occasionally, relief of pain resulted without an appreciable change in the degree of cyanosis. Intermittent claudication proved rather refractory. Partial relief was obtained often but complete relief rarely. Small chronic ulcers became less painful and tended to heal slowly. Neither frank gangrene nor complicating osteomyelitis was affected. The presence of even large varicose veins did not interfere with the treatment or its results. In patients who responded most favorably, sweating increased, the skin became pink, the temperature of the skin rose and pain and paresthesia disappeared. As it is important not to overrate a method which is still in the experimental stage, we have graded our results as conservatively and judiciously as possible.

Arteriosclerosis Obliterans.—Tables 1, 2 and 3 present the essential data in this group of thirty-nine cases. Our policy at first was to test the method wholly without prejudice. This explains the inclusion in table 3 of cases in which the likelihood of securing beneficial results from conservative methods of treatment was extremely small. The number of failures was therefore somewhat larger than it would be under ordinary circumstances. It should be noted that absence of the posterior tibial pulse was a conspicuous feature in the cases in which poor results were obtained and that diabetes mellitus bore no relationship to the outcome of the treatment.

Thrombo-Angiitis Obliterans.—Table 4 epitomizes the data in a group of eight cases. Only two patients (25 per cent) obtained much relief.

TABLE 1.—*Data in Cases of Arteriosclerosis Obliterans (Good Results in 33 per Cent of Thirty-Nine Cases)* *

				Symptoms				Objective Manifestations						Pulses						
Case	Age	Sex	Reason for Treatment	Coldness	Paresthesias	Rest Pain	Claudication	Coldness	Permanent Changes in Color	Postural Changes in Color	Trophic Changes	Ulceration	Gangrene	Dorsalis Pedis	Posterior Tibial	Popliteal	Femoral	Amount of Treatment, Hours	Results	Other Pathologic Conditions
1	60	M	Refractory ulcer on right great toe	+	+	0	0	+	+	+	+	+	0	0 +	0 +	+ +	+ +	145	Ulcer healed rapidly	None
2	58	M	Refractory ulcer on right great toe	0	0	0	0	+	+	0	+	+	0	+ 0	+ +	+ +	+ +	75	Ulcer healed; foot became warmer	Diabetes mellitus
3	60	M	Cold foot; paresthesias	+	+	0	0	+	+	0	+	0	0	+ —	+ —	+ —	+ —	40	Foot became warmer; paresthesia diminished	Diabetes mellitus; left leg previously amputated
4	58	F	Gangrene and chronic infection of right great toe	+	+	+	0	0	+	0	+	+	+	+ 0	0 0	+ +	+ +	100	Toe sloughed; stump healed promptly	Diabetes mellitus
5	62	M	Intermittent claudication	+	+	0	+	+	+	0	+	0	0	0 0	0 +	+ +	+ +	50	Relieved of all distress	Pes planus
6	61	M	Refractory digital ulcer; cold foot	+	0	0	0	+	+	+	+	+	0	0 —	0 —	0 —	+ —	105	Ulcer healed promptly; foot became warmer	Diabetes mellitus; left leg previously amputated
7	68	M	Cold feet	+	+	0	0	+	0	0	0	0	0	+ +	+ +	+ +	+ +	10	Feet became warmer	None
8	67	M	Cold, painful feet; intermittent claudication	+	+	+	+	+	+	+	+	0	0	+ 0	0 0	+ 0	+ +	200	Feet became warmer; claudication disappeared	None
9	59	M	Stumps of two previously amputated toes would not heal	0	0	+	0	0	0	0	0	+	+	0 0	+ +	+ +	+ +	85	Healing greatly accelerated	Diabetes mellitus
10	61	M	Cold feet and intermittent claudication	+	+	0	+	+	+	0	+	0	0	0 0	0 0	+ +	+ +	150	Feet became warmer; patient could walk twice as far	Average blood pressure, 180 systolic and 130 diastolic
11	80	M	Painful, red feet	+	+	+	?	+	+	0	+	0	0	0 0	0 0	+ +	+ +	50	Pain relieved; color unchanged	Coronary arteriosclerosis; fatal pneumonia
12	78	M	Stump of previously amputated left great toe would not heal	+	+	+	0	+	+	0	+	+	0	— 0	— 0	— +	— +	20	Stump healed rapidly	Diabetes mellitus; right leg previously amputated
13	71	M	Painful, red feet	+	+	+	0	+	+	0	0	0	0	+ 0	+ 0	+ +	+ +	120	Pain relieved; color not appreciably changed	Average blood pressure, 200 systolic and 120 diastolic; varicose veins

* In this and the following tables a pulse is indicated as present (+) or absent (0), and in each case the top row represents the right side and the bottom the left side.

TABLE 2.—*Data in Cases of Arteriosclerosis Obliterans (Fair Results in 25 per Cent of Thirty-Nine Cases)*

Case	Age	Sex	Reason for Treatment	Symptoms: Coldness	Paresthesias	Rest Pain	Claudication	Objective Manifestations: Coldness	Permanent Changes in Color	Postural Changes in Color	Trophic Changes	Ulceration	Gangrene	Pulses: Dorsalis Pedis	Posterior Tibial	Popliteal	Femoral	Amount of Treatment, Hours	Results	Other Pathologic Conditions
1	66	M	Refractory ulcer on right great toe	+	+	+	+	+	+	0	+	+	0	0 0	0 0	+ +	+ +	240	Feet became warmer; pain diminished, but right leg was amputated later (infection)	Coronary arteriosclerosis
2	71	M	Painful, cold feet; claudication; refractory ulcer on toe	+	+	+	+	+	+	+	+	+	0	0 0	0 0	+ +	+ +	110	Feet became warmer; pain diminished; ulcer healed slowly	None
3	70	M	Painful refractory ulcer on right foot	+	+	+	0	+	0	0	+	+	0	+ —	+ —	+ —	+ —	150	Ulcer healed, but gangrene 6 mo. later required amputation of leg	Diabetes mellitus; left leg previously amputated
4	72	M	Painful ulcer on right heel	+	+	+	0	+	+	+	+	+	0	0 —	0 —	+ —	+ —	140	Ulcer healed, but gangrene 2 mo. later required amputation of leg	Diabetes mellitus; left leg previously amputated
5	42	F	Stump of previously amputated left great toe would not heal	0	0	+	0	0	0	0	0	+	+	— +	— +	— +	— +	50	Healing accelerated	Diabetes mellitus; right leg previously amputated
6	74	M	Cold, red, painful foot; refractory ulcer on toe	+	+	+	0	+	+	+	+	+	0	— 0	— +	— 0	— +	220	Foot remained cold and red, but pain was relieved; ulcer healed	Pulmonary emphysema; right leg previously amputated
7	60	M	Intermittent claudication	?	+	+	+	?	+	+	+	0	0	0 0	0 0	0 0	+ +	100	Claudication somewhat lessened; feet became warmer	None
8	54	M	Stump of previously amputated toe would not heal	+	+	0	0	+	+	+	+	+	0	+ +	0 0	+ +	+ +	25	Healing accelerated temporarily	Diabetes mellitus
9	73	F	Stump of previously amputated toe would not heal	+	+	+	0	+	0	0	0	+	0	+ 0	0 0	+ +	+ +	100	Healing accelerated	Diabetes mellitus
10	53	M	Intermittent claudication	+	+	0	+	+	+	+	?	0	0	0 +	+ +	+ +	+ +	100	Exercise tolerance doubled; feet became warmer	None

				Symptoms				Objective Manifestations					Pulses						
Case	Age	Sex	Reason for Treatment	Coldness	Paresthesias	Rest Pain	Claudication	Coldness	Permanent Changes in Color	Postural Changes in Color	Trophic Changes	Ulceration	Gangrene	Dorsalis Pedis	Posterior Tibial	Popliteal	Femoral	Amount of Treatment, Hours	Results	Other Pathologic Conditions
1	55	M	Gangrene and infection of one toe	+	+	+	+	+	+	0	+	+	+	0 0	0 0	+ +	+ +	207	Gangrene spread, requiring amputation of leg; stump treated unsuccessfully	Diabetes mellitus
2	75	M	Cold, painful feet; intermittent claudication	+	+	+	+	+	+	0	+	0	0	0 0	0 0	+ +	+ +	30	Began to improve, but cuffs caused so much discomfort that patient went home	Coronary arteriosclerosis
3	75	M	Painful, red left foot	+	+	+	+	+	+	+	+	0	0	+ 0	0 0	+ +	+ +	45	Gangrene of two toes developed during treatment, necessitating amputation of leg	None
4	72	M	Painful, red right foot, with small ulcer	+	+	+	+	+	+	0	+	+	0	0 +	0 +	+ +	+ +	80	Ulcer enlarged; gangrene developed	Coronary arteriosclerosis
5	66	M	Large refractory ulcer on left heel	+	+	+	0	+	+	0	+	+	0	+ +	0 0	+ +	+ +	35	Ulcer enlarged and became more painful	Diabetes mellitus
6	75	M	Claudication	+	0	0	+	+	0	0	+	0	0	0 0	0 0	+ +	+ +	22	No improvement; treatment discontinued because cuffs blistered thighs	Coronary arteriosclerosis
7	72	M	Severe intermittent claudication	+	+	0	+	+	+	+	+	0	0	0 0	0 0	+ +	+ +	80	No improvement	Coronary arteriosclerosis
8	52	M	Cold feet; intermittent claudication	+	+	+	+	0	+	+	0	0	0	+ 0	0 0	+ +	+ +	15	Treatment produced pain in feet; cuffs caused discomfort	Latent syphilis
9	74	M	Gangrene of dorsum of left foot	+	+	+	+	+	+	+	+	+	+	+ 0	+ 0	+ +	+ +	60	Gangrene spread, necessitating amputation	None
10	68	M	Stump of previously amputated toe would not heal	+	+	+	0	+	+	0	0	+	+	0 0	0 0	+ +	+ +	80	Healing slightly accelerated; pain unrelieved	Diabetes mellitus
11	65	M	Stump of previously amputated right leg would not heal	—	—	—	—	—	—	—	—	—	—	— —	— —	+ —	+ —	35	No improvement	Average blood pressure, 200 systolic and 108 diastolic; left leg previously amputated
12	62	M	Red, painful right foot, with two small ulcers	?	+	+	+	+	+	0	+	+	0	0 +	0 0	+ +	+ +	95	No relief from pain; ulcers became larger	None
13	55	F	Stump of previously amputated toe would not heal	+	+	+	0	+	+	0	+	+	+	— 0	— 0	— 0	— +	35	Treatment caused pain; healing not accelerated	Diabetes mellitus; right leg previously amputated
14	68	F	Left foot cold and painful; dry gangrene of one toe	+	+	+	0	+	+	0	+	+	+	— 0	— 0	— 0	— +	90	Foot became warmer, but an ulcer appeared on heel; amputation will be necessary	Average blood pressure, 250 systolic and 110 diastolic; uremia; diabetes; right leg previously amputated
15	58	M	Gangrenous, painful toe	+	+	+	0	+	+	0	+	+	+	0 —	0 —	+ —	+ —	20	Treatment increased pain and was discontinued; toe amputated successfully	Left leg previously amputated
16	68	F	Cold, red, painful feet, with ulcers on two toes	+	+	+	0	+	+	+	+	+	0	0 0	0 0	0 0	+ +	35	Treatment increased pain; feet remained cold; ulcers unaffected	Diabetes mellitus

TABLE 4.—*Data in Cases of Thrombo-Angiitis Obliterans*

				Symptoms				Objective Manifestations						Pulses								
Case	Age	Sex	Reason for Treatment	Coldness	Paresthesias	Rest Pain	Claudication	Coldness	Permanent Changes in Color	Postural Changes in Color	Phlebitis	Trophic Changes	Ulceration or Gangrene	Radial	Ulnar	Dorsalis Pedis	Posterior Tibial	Popliteal	Femoral	Amount of Treatment, Hours	Results	Other Pathologic Conditions
1	35	M	Rest pain; intermittent claudication	0	+	+	+	+	+	+	+	0	0	+ 0	0 0	0 0	0 0	+ +	+ +	195	Good; rest pain and claudication greatly relieved	None
2	44	M	Ulcer on index finger of left hand; rest pain in hand	+	+	+	0	+	+	+	0	+	+	+ +	+ 0	0 0	+ +	+ +	+ +	128	Good; pain relieved; ulcer healed rapidly	None
3	35	M	Small ulcer on left foot; rest pain	+	+	+	+	+	+	+	+	+	+	+ +	+ +	0 0	0 0	+ 0	+ +	155	Fair; pain somewhat relieved; ulcer unaffected	None
4	39	M	Intermittent claudication	0	0	+	+	0	+	+	0	0	0	+ +	+ +	0 0	0 0	0 0	+ +	100	Poor; no relief from claudication	History of coronary occlusion
5	47	M	Painful, red feet; intermittent claudication	+	+	+	+	+	+	+	+	+	0	0 +	+ 0	0 0	0 0	0 0	+ +	185	Poor; no improvement	None
6	56	M	Painful, red feet; stump of previously amputated toe not healing	+	+	+	+	+	+	+	+	+	+	+ +	0 0	0 0	0 0	0 +	+ +	300	Poor; some relief from pain; ulcer grew smaller but enlarged again later	None
7	43	M	Painful, red feet; ulcers on two toes of left foot	+	+	+	+	+	+	+	+	+	+	0 +	+ +	+ 0	0 0	0 0	+ +	117	Poor; pain unrelieved; ulcers enlarged	None
8	39	M	Intermittent claudication	+	+	+	+	+	+	+	+	+	0	+ +	+ ?	0 0	0 0	? ?	+ +	100	Poor; no improvement	None

Case	Age	Sex	Reason for Treatment	Symptoms: Coldness	Symptoms: Paresthesias	Symptoms: Rest Pain	Symptoms: Claudication	Objective Manifestations: Coldness	Objective Manifestations: Permanent Changes in Color	Objective Manifestations: Postural Changes in Color	Objective Manifestations: Trophic Changes	Objective Manifestations: Ulceration	Objective Manifestations: Gangrene	Pulses: Radial	Pulses: Ulnar	Pulses: Dorsalis Pedis	Pulses: Posterior Tibial	Pulses: Popliteal	Pulses: Femoral	Amount of Treatment, Hours	Results	Other Pathologic Conditions
1	34	F	Occlusion of right popliteal artery 3 mo. earlier; gangrenous ulcer occupying one half of sole of foot	+	+	+	0	+	+	0	+	+	+	+	+	0	0	0	+	87	Good; ulcer healed rapidly; foot became warm and painless; no return of pulses	Severe rheumatic heart disease; auricular fibrillation
														+	+	+	+	+	+			
2	60	F	Occlusion of abdominal aorta at bifurcation 8 mo. earlier; severe bilateral claudication	0	0	0	+	+	0	0	0	0	0	+	+	0	0	0	0	122	Good; claudication greatly relieved	Average blood pressure, 210 systolic and 95 diastolic; generalized arteriosclerosis
														+	+	0	0	0	0			
3	45	F	Occlusion of right brachial artery a few hours earlier; cold, painful hand	+	+	+	0	+	+	0	0	0	0	0	0	+	+	+	+	12	Good; hand became warm; pain relieved; small radial pulse appeared	Mild rheumatic heart disease; auricular fibrillation
														+	+	+	+	+	+			
4	54	M	Occlusion of left popliteal artery 5 mo. earlier; severe claudication and rest pain	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	346	Good; after 136 hrs. of treatment pain nearly abolished; after 210 hrs., 4 mo. later, pain abolished completely; foot remained somewhat cool	Coronary arteriosclerosis; auricular fibrillation; thromboangiitis obliterans (?)
														+	+	0	0	0	+			
5	60	M	Occlusion of left popliteal artery an hour earlier; cold, painful foot	+	+	+	0	+	+	0	0	0	0	+	+	+	0	+	+	32	Good; foot became warmer than its fellow; pain and numbness disappeared	Average blood pressure, 190 systolic and 110 diastolic; history of coronary occlusion
														+	+	0	0	0	+			
6	86	M	Occlusion of right popliteal artery 24 hrs. earlier; red, painful foot	+	+	+	0	+	+	+	0	0	0	—	—	0	0	0	+	45	Good; foot became warmer and practically painless; patient died of pneumonia	Coronary arteriosclerosis
														—	—	0	+	+	+			
7	18	M	Ligation of right femoral artery just below first perforating branch 1 yr. earlier; claudication; cold foot	+	+	0	+	?	0	0	0	0	0	—	—	+	+	0	+	100	Good; could walk twice as far; paresthesias disappeared; foot warmer	None
														—	—	+	+	0	+			
8	47	M	Occlusion of arteries of three fingers of right hand 1 mo. earlier; cold, numb fingers	+	+	0	0	+	+	0	0	0	0	+	+	0	+	+	+	100	Good; fingers became much warmer; paresthesias practically disappeared	None
														+	+	+	+	+	+			
9	65	M	Occlusion of right brachial artery 3 weeks earlier; cold, numb hand	+	+	0	0	+	+	0	0	0	0	+	0	+	+	+	+	18	Fair; hand became warmer, but patient refused further treatment	None
														+	+	+	+	+	+			
10	76	F	Occlusion of right popliteal artery 5 days earlier; cold, painful foot	+	+	+	0	+	+	0	0	0	0	—	—	0	0	0	+	24	Fair; foot warmer; pain not entirely relieved	Coronary arteriosclerosis; old hemiplegia
														—	—	+	+	+	+			
11	54	M	Occlusion of left popliteal artery 1 mo. earlier; cold, discolored painful foot	+	+	+	0	+	+	+	0	0	0	—	—	+	+	+	+	25	Poor; leg became warmer, but discoloration deepened; amputation necessary	Mild rheumatic heart disease; auricular fibrillation
														—	—	0	0	0	+			
12	65	F	Occlusion of left brachial artery an hour earlier; cold, painful hand	+	+	+	0	+	+	0	0	0	0	+	+	—	—	—	—	7	Poor; no relief of pain or change of color, but patient died in circulatory collapse 12 hours later	Coronary arteriosclerosis; mesenteric thrombosis
														0	0	—	—	—	—			

TABLE 6.—*Data in Cases of Miscellaneous Disorders*

				Symptoms			Objective Manifestations						Pulses								
Case	Age	Sex	Reason for Treatment	Coldness	Paresthesias	Rest Pain	Coldness	Permanent Changes in Color	Postural Changes in Color	Trophic Changes	Ulceration	Gangrene	Radial	Ulnar	Dorsalis Pedis	Posterior Tibial	Popliteal	Femoral	Amount of Treatment, Hours	Results	Other Pathologic Conditions
1	32	M	Feet frozen several weeks earlier; stumps of several toes which had sloughed were healing poorly	0	+	+	0	+	0	0	+	0	+ +	+ +	+ +	+ +	+ +	+ +	30	Healing somewhat accelerated	None
2	34	M	Hand exposed to severe cold a few hours earlier; severe pain	0	+	+	0	+	0	0	0	0	+ +	+ +	+ +	+ +	+ +	+ +	4	Pain relieved completely	None
3	57	F	Raynaud's disease; arteriosclerosis obliterans; small ulcer on one toe	+	+	+	+	+	0	+	+	+	+ +	+ +	0 0	+ +	+ +	+ +	5	No improvement; pain aggravated by treatment	Coronary arteriosclerosis; average blood pressure, 180 systolic and 100 diastolic
4	39	M	Raynaud's disease; ulcer on tip of right index finger	+	+	+	+	+	0	+	+	0	+ +	+ +	+ +	+ +	+ +	+ +	42	Pain somewhat relieved only during actual treatment; healing of ulcer accelerated	Peptic ulcer of duodenum
5	25	M	Arterial insufficiency after local anesthesia of right index finger 1 week earlier; small ulcer on finger tip	+	+	+	+	+	0	0	+	0	+ +	+ +	+ +	+ +	+ +	+ +	26	Pain relieved; finger became warmer; ulcer healed slowly	None
6	16	M	Refractory ulcers of uncertain origin on both legs (varicose)	0	0	0	0	0	0	0	+	0	+ +	+ +	+ +	+ +	+ +	+ +	90	No improvement	Chronic glomerular nephritis
7	54	M	Refractory ulcers on both feet, probably due to disease of spinal cord	0	0	0	0	+	+	+	+	0	— —	— —	0 0	+ +	+ +	+ +	55	No improvement	Congenital malformation of spinal cord (?)
8	36	M	Obscure vasomotor disturbance	0	+	+	+	0	+	0	0	0	+ +	+ +	+ +	+ +	+ +	+ +	120	No improvement	Generalized arteriosclerosis and capillary fibrosis; chronic diffuse nephritis (necropsy)
9	28	M	Three toes of left foot frozen several months earlier; severe rest pain; small ulcer on great toe	+	+	+	+	+	+	+	+	0	+ +	+ +	+ 0	+ 0	+ +	+ +	5	No improvement; pain aggravated by treatment; foot amputated	Histologic appearance not that of thrombo-angiitis obliterans

Sudden Occlusion of a Major Artery.—Although this is not a progressive obliterative process and a patient may recover unaided even from occlusion of the abdominal aorta at its bifurcation, it is nevertheless obvious that passive vascular exercise increases enormously the likelihood and rapidity of restitutio in integrum. This noteworthy achievement is in itself sufficient recompense for all the time and effort which have been expended in developing the method. Table 5 summarizes our experience in twelve cases. In spite of the fact that in most of these cases treatment was not started until long after the occlusion had occurred, the results were excellent in all but the last two cases, and in those cases the condition was virtually hopeless from the beginning. Case 1 deserves particular notice. Three months after sudden occlusion of the right popliteal artery, all the characteristic manifestations of arterial insufficiency, including gangrenous ulceration of half the sole of the foot, were present. After eighty-seven hours of treatment were given, recovery was practically complete.

Miscellaneous Conditions.—Reference to table 6 shows that passive vascular exercise is not a panacea and should not be employed indiscriminately. In this group only the immediate consequences of frostbite (case 2) and arterial insufficiency secondary to local anesthesia (case 5) proved amenable to treatment by this method.

SUMMARY

This report is, in its entirety, a summary. Our experience indicates, as does that of many other observers, that if collateral pathways are potentially adequate, passive vascular exercise helps to reestablish the circulation in an extremity which has been deprived of part of its blood supply by obliterative arterial disease.

EFFECT OF MAGNESIUM ON VASCULAR SPASM IN RATS

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In a previous study it was demonstrated that the magnesium ion is capable of preventing the contraction of smooth muscle both in vitro and in vivo.[1] In a large percentage of guinea-pigs passively sensitized to horse serum an intravenous injection of magnesium chloride just prior to the injection of a shocking dose of horse serum prevented the appearance of anaphylactic shock. Similarly, the addition of magnesium chloride in isotonic amounts to the fluid of the Dale bath in which were suspended uterine strips from sensitized guinea-pigs prevented the contraction of the strips when antigen was added.

EXPERIMENTS

The present studies were concerned with the effect of the magnesium ion on the contraction of the smooth muscle of the vascular system in the intact animal. White rats (Wistar strain) were utilized as the experimental animals, and the systolic blood pressure was used as an index of the presence or absence of vascular spasm. The technic employed for the determination of the systolic blood pressure in the rat was the indirect method of Griffith.[2]

Technic of Measuring the Systolic Blood Pressure.—The apparatus employed in determining the rat's blood pressure is illustrated in figure 1. The rat is kept under constant and complete ether anesthesia during the period of determination, for it has been found that with the animal under light anesthesia his movements produce fluctuations in the blood pressure. The dorsum of the hindleg is shaved, care being taken to avoid traumatizing the skin, as this produces multiple thromboses of small vessels and interferes with the determination of the blood pressure.

The blood pressure cuff is made from a ring of thin elastic sheet rubber with an internal diameter of about ½ inch (1.3 cm.) and a width of about ⅛ inch (1 cm.). The cuff is reenforced on the outer side with several thicknesses of

Aided by a grant from Mead Johnson & Co.

A preliminary report of this study was presented before the Society for Pediatric Research, Atlantic City, N. J., May 5, 1936.

From the Department of Pediatrics, the School of Medicine, the University of Pennsylvania, and the Children's Hospital of Philadelphia.

1. Rubin, M. I.: Studies on Anaphylaxis (unpublished), presented before the Pediatric Research Society, Atlantic City, N. J., May 3, 1932.

2. Griffith, J. Q., Jr.: Indirect Method for Determining Blood Pressure in Small Animals, Proc. Soc. Exper. Biol. & Med. **32**:394, 1934.

sheet rubber to prevent bulging. The cuff is connected to one arm of a Y tube. The second arm of the Y tube is connected to a standard mercury manometer and the third arm to a rubber compression bulb. The rubber bulb is kept in a wooden box, where constant pressure may be applied with a screw clamp.

The leg of the rat, after the cuff is applied to the thigh, is supported on the microscopic stage with plasticine. The small cutaneous vessels on the dorsum of the shaved leg are observed under liquid petrolatum with the low power objective of the microscope, indirect illumination being used. Cutaneous vessels in which the flow of blood is very rapid are chosen for observation. The movement of the column of blood in suitable vessels is continuous and so rapid that individual blood cells cannot be distinguished.

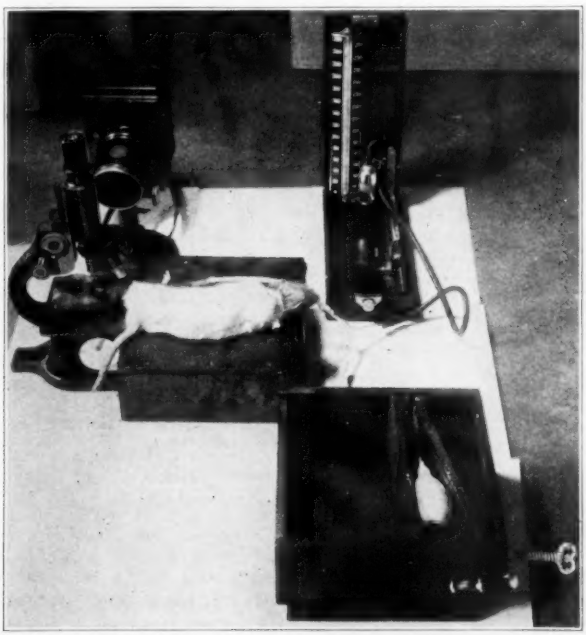

Fig. 1.—The apparatus employed in determining the systolic blood pressure of the rats.

Pressure is applied to the rubber bulb in increments of from 2 to 4 mm. of mercury while the vessels are kept under constant observation. The pressure necessary to stop the flow of blood in the small vessels is taken as the systolic blood pressure of the animal. The average of three consecutive readings is considered the final reading, provided the individual readings do not vary more than 15 mm. of mercury. After many readings have been taken, edema of the leg often develops and renders it unsuitable for further observation at the time. If further readings are required, the other hindleg is used.

Griffith [2] has shown that the readings of the systolic blood pressure of rats taken by this indirect method are comparable with the direct readings obtained

by inserting a needle into a large artery. The values obtained by us for the systolic blood pressure of normal rats are similar to those found by Griffith[3] and co-workers and by Leiter,[4] who also measured the blood pressure by cannulization of the arteries.

Experimental Data.—Persistent vascular spasm with resultant hypertension was produced by the parenteral administration of ergotamine tartrate.[5] There is adequate evidence to show that this drug produces intense vasoconstriction. Herrick[6] found that from 0.5 to 1 mg. of this drug given parenterally markedly reduced the blood flow in the femoral artery of the dog and raised the systemic blood pressure. Pool and Nason[7] by direct observation with the microscope demonstrated constriction of the dural and the cutaneous arteries after the administration of ergotamine. Lewis[8] has shown that there is intense, per-

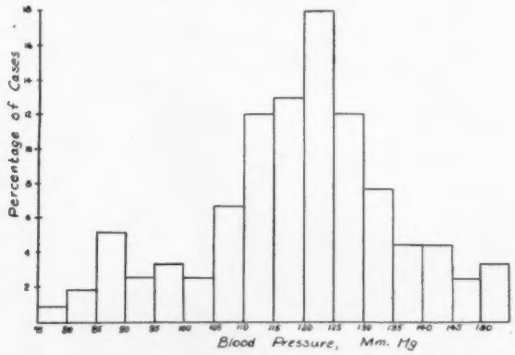

Fig. 2.—The distribution of blood pressure readings for one hundred and seventeen normal rats.

3. Griffith, J. Q., Jr.; Jeffers, W. A., and Lindauer, M. A.: A Study of the Mechanism of Hypertension Following Intracisternal Kaolin Injection in Rats: Leucocytic Reaction and Effect on Lymphatic Absorption, Am. J. Physiol. **113**: 284, 1935.

4. Leiter, L.: The Nonspecific Rôle of Pressor Substances in the Plasma of Hypertensive Patients, Arch. Int. Med. **57**:729 (April) 1936.

5. The ergotamine tartrate used in these experiments was supplied by the Sandoz Chemical Works, Inc.

6. Herrick, J. F.: Effect of Ergotamine Tartrate on Blood Flow and Blood Pressure in the Femoral Artery of the Dog, Proc. Soc. Exper. Biol. & Med. **30**: 271, 1933.

7. Pool, J. L., and Nason, G. I.: Cerebral Circulation: The Comparative Effect of Ergotamine Tartrate on the Arteries in the Pia, Dura and Skin of Cats, Arch. Neurol. & Psychiat. **33**:276 (Feb.) 1935.

8. Lewis, T., and Gelfand, B.: The Manner in Which Necrosis Arises in the Fowl's Comb Under Ergot Poisoning, Clin. Sc. **2**:43, 1935.

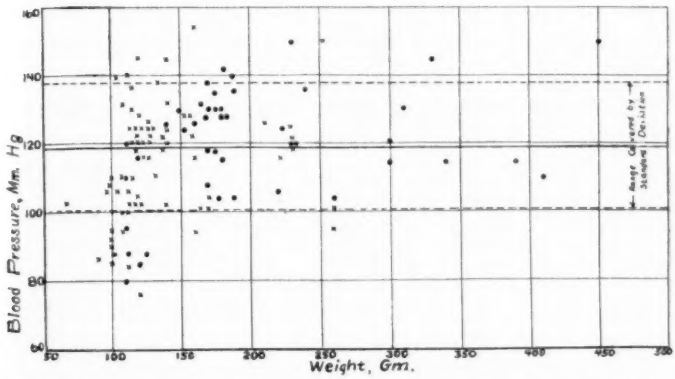

Fig. 3.—The normal blood pressure levels for rats of both sexes and different weights. The black circles indicate males and the crosses females.

Data on the Systolic Blood Pressure of Rats

Systolic Blood Pressure, Mm. Hg	Normal Rats			Rats Given Ergotamine Tartrate					
				Normal Diet			Magnesium Diet		
	Number of Rats	Cumulative Number of Rats	Cumulative Percentage of Rats	Number of Rats	Cumulative Number of Rats	Cumulative Percentage of Rats	Number of Rats	Cumulative Number of Rats	Cumulative Percentage of Rats
75- 79	1	1	0.86	..	..	....	..	..	....
80- 84	2	3	2.55	..	..	....	..	..	....
85- 89	6	9	7.70	..	..	....	..	..	....
90- 94	3	12	10.20	..	..	....	1	1	1.5
95- 99	4	16	13.70	..	..	....	..	..	....
100-104	3	19	16.30	..	..	....	8	9	13.8
105-109	8	27	23.00	..	..	....	7	16	25.4
110-114	14	41	35.80	..	..	....	4	20	30.8
115-119	15	56	47.80	..	..	....	7	27	42.8
120-124	16	77	65.70	..	..	....	13	40	61.5
125-129	14	91	77.80	..	..	....	6	46	73.0
130-134	9	100	85.50	2	2	3.2	6	52	82.5
135-139	5	105	89.90	..	..	....	3	55	87.3
140-144	5	110	94.00	1	3	4.8	5	60	92.3
145-149	3	113	96.50	..	..	....	1	61	94.0
150-154	4	117	100.00	1	4	6.4	..	..	....
155-159	..	..	.....	1	5	7.8	..	..	....
160-164	..	..	.....	7	12	19.1	1	62	95.5
165-169	..	..	.....	4	16	25.4	..	..	....
170-174	..	..	.....	7	23	36.5	1	63	97.0
175-179	..	..	.....	9	32	50.9	2	65	100.0
180-184	..	..	.....	3	35	55.5	..	..	....
185-189	..	..	.....	2	37	58.6	..	..	....
190-194	..	..	.....	6	43	68.3	..	..	....
195-199	..	..	.....	2	45	71.5	..	..	....
200-204	..	..	.....	7	52	82.5	..	..	....
205-209	..	..	.....	1	53	84.2	..	..	....
210-214	..	..	.....	2	55	87.5	..	..	....
215-219	..	..	.....	5	60	95.2	..	..	....
220-224	..	..	.....	1	61	96.8	..	..	....
225-229	..	..	.....	1	62	97.8	..	..	....
230-234	..	..	.....	2	63	100.0	..	..	....
	Average systolic blood pressure: 119 mm. Hg ± 19 (S. D.)			Average systolic blood pressure: 185 mm. Hg ± 23 (S. D.)			Average systolic blood pressure: 121 mm. Hg ± 18 (S. D.)		

sistent vasoconstriction in the comb of the fowl treated with ergotoxine, the duration of the vascular spasm varying with the dose of the drug.

Blood Pressure of Normal Rats.—The accompanying table and figures 2 and 3 show the variations in the systolic blood pressure of one hundred and seventeen normal rats raised on our colony diet.

Composition of Diet	Gm.
Whole ground wheat	660
Whole ground corn	680
Whole milk powder	420
Linseed oil meal	140
Alfalfa meal	40
Liver	40
Calcium carbonate	10
Sodium chloride	10
Dried brewer's yeast	40

It is apparent that weight and sex do not influence the blood pressure significantly. The range of these normal blood pressures was from 78 to 154 mm. of mercury, the average being 119, with a standard deviation of ± 19. It is interesting that 69 per cent of the values obtained by Leiter, who used the direct method, fell between 101 and 140 mm. of mercury and that the range of blood pressures covered by our standard deviation, which included 68.26 per cent of the total readings, was between 100 and 138 mm. of mercury. Repeated readings for the same rat showed no significant fluctuations.

Blood Pressure of Rats Given Injections of Ergotamine Tartrate.—One hundred and twenty-eight rats, weighing from 75 to 100 Gm. at the onset of the experiment, were divided into two equal groups on the basis of weight and sex. One group was kept on the diet for the normal colony; the other group was given the same diet, to which 2 per cent of magnesium carbonate by weight was added. Magnesium carbonate when fed at this level did not produce diarrhea in the rats. The stools, however, were soft and pasty. After the rats had been on these diets for about three weeks the blood pressure was determined, and then the animals in both groups were given subcutaneous injections of 15 mg. of ergotamine tartrate per kilogram of body weight. Some animals in each group received but one injection, others received two injections and still others received three injections at weekly intervals. In no case was the subsequent blood pressure reading recorded prior to one week after the last injection. In some cases the readings were not taken until from two to five weeks after the last injection. In every instance, however, the readings were paired, the animals on different diets being studied at the same time.

The accompanying table and figure 4 show the blood pressure levels of the rats on the two diets which were given ergotamine. Ninety-four per cent (or sixty-one) of the sixty-five animals on the magnesium diet had a systolic blood pressure which was well within the normal range. Ninety-three and six-tenths per cent (or fifty-nine) of the sixty-three animals on the normal diet, however, had hypertension.

In one experiment (figs. 5 and 6) after the effect of ergotamine on two groups of rats had been determined the diets were reversed. The group of animals in which hypertension had developed when they were given the normal diet responded with a fall in blood pressure to within normal range when placed on

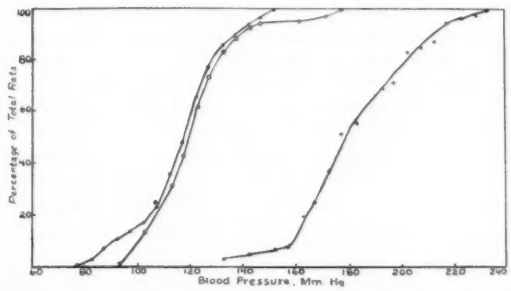

Fig. 4.—Cumulative frequency curves of the blood pressure readings for three groups of rats. The black circles indicate the readings for one hundred and seventeen normal rats which were fed a normal diet; the white circles indicate the readings for sixty-five rats which were fed a magnesium-containing diet and given injections of ergotamine tartrate, and the crosses indicate the readings for sixty-three rats fed a normal diet which were given injections of ergotamine tartrate.

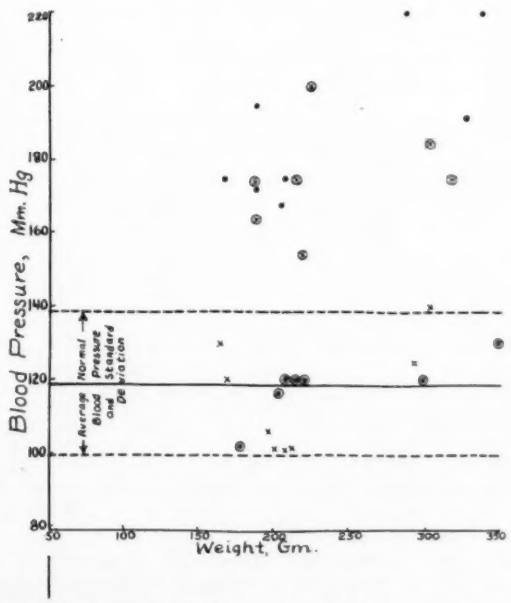

Fig. 5.—The blood pressure readings for rats which were given injections of ergotamine tartrate and which were maintained on different diets, the effect of reversing the diets being noted. The solid black circles represent readings for rats on a normal diet; the white circles with a solid black center, the readings for rats changed from a normal to a magnesium-containing diet; the crosses, the readings for rats fed on a magnesium-containing diet, and the white circles with a cross in the center, the readings for rats changed from a magnesium-containing to a normal diet.

the magnesium diet. Conversely, the animals which had failed to show hypertension while on the magnesium diet responded with a rise in blood pressure when magnesium was withdrawn from the diet. These changes in blood pressure were produced forty days after the injections of ergotamine tartrate were given. Readings were taken for another thirty days, and the effects were still operative. The implication of this experiment, in which the reversal in the blood pressure readings could be effected as long as forty days after the injection of three doses of ergotamine tartrate, is that the effect of magnesium is not to rid the body of the ergotamine but to hold in abeyance the vasoconstricting action of the drug.

Effect of Ergotamine on Growth and Development.—The weight curves for both groups of rats which had been given ergotamine, i. e., those on the normal diet and those on the magnesium diet, were below the average weight for our

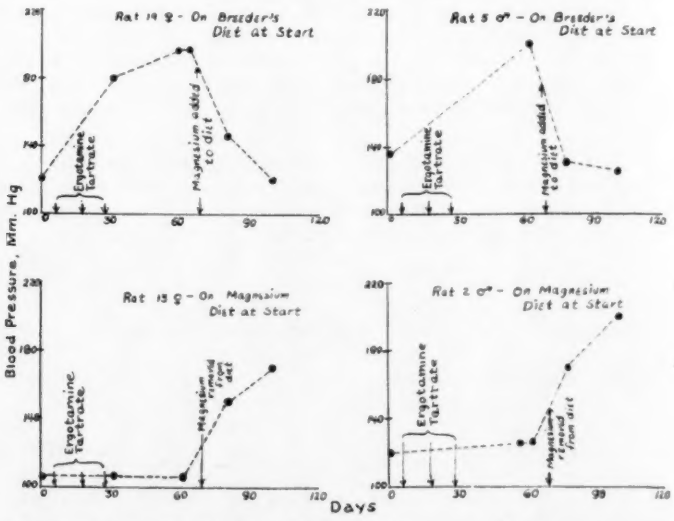
Rat 19 ♀ - On Breeder's Diet at Start
Rat 5 ♂ - On Breeder's Diet at Start
Rat 13 ♀ - On Magnesium Diet at Start
Rat 2 ♂ - On Magnesium Diet at Start
Ergotamine Tartrate
Magnesium added to diet
Magnesium removed from diet
Blood Pressure, Mm. Hg
Days

Fig. 6.—Graphs showing the effect of adding magnesium to and removing magnesium from the diet.

normal colony rats. A comparison of weight curves showed no difference between the animals on the normal diet and the animals on the magnesium diet sixty days after the last injection of ergotamine tartrate.

Because diets high in magnesium have been used to produce rickets in the rat,[9] roentgenographic studies were carried out on our animals to determine whether or not osseous changes had occurred. No detectable differences in the density or the structure of the bones were evident in the roentgenograms of the rats fed the normal diet and rats fed the magnesium diet.

Pathologic Observations.—Peripheral gangrene of the tail developed in almost all the animals on both diets which were given ergotamine. This lesion was

9. Meyer zu Hörste, G.: Magnesium Rachitis, Monatschr. f. Kinderh. **54**: 195, 1932; Klin. Wchnschr. **11**:1796, 1932.

similar to that described by McGrath [10] in the rat's tail and by Lewis and Gelfand [8] in the fowl's comb. The tails of the rats in this experiment were studied by Dr. Arthur D. Waltz, pathologist for the Children's Hospital, and the following report was made:

"Sections of the gangrenous tails show the epidermis shrunken and the areolar connective tissue pale, containing small clear fluid spaces. The muscle fibers are granular, the striations lost and the muscles degenerated. Practically all the arterioles contain thrombi in different stages of formation; those of more recent origin consist largely of fibrin containing sinuses filled with erythrocytes. These vessel walls show areas of the intima denuded of lining endothelium; the appearance of the rest of the wall is normal. Other thrombi are well organized and partially canalized. Occasionally a vessel and its contained thrombus are definitely degenerated. All other blood vessels, from the smallest capillaries to the comparatively large vessels, are greatly dilated and packed with red blood cells. There are no noteworthy changes in the bone."

The urine of both groups of rats which were given ergotamine contained albumin. No organized sediment was present.

COMMENT

There is ample evidence that ergotamine tartrate produces vasoconstriction in the intact animal. Since the early description of the action of ergot by Dale,[11] in which he stated that one of the major effects of this drug is direct stimulation of the smooth musculature of arteries producing contraction, many other workers have confirmed this observation.[12] This vascular spasm, we believe, is directly responsible for the hypertension produced in our rats. A rise in the systolic blood pressure following the administration of ergotamine tartrate has been observed by Herrick [6] in the dog, by Pool and Nason [7] in the cat and by Pool, von Storch and Lennox [13] in human beings. While the rises in the systolic blood pressure reported by these workers were not of as great magnitude as those reported in this paper, it should be emphasized that the doses of ergotamine which they employed were much smaller than those utilized in our experiment.

Studies by the general physiologist show that the magnesium ion is capable of producing relaxation of the smooth muscle fiber. Thus Tren-

10. McGrath, E. J. G.: Experimental Peripheral Gangrene: Effect of Estrogenic Substance and Its Relation to Thrombo-Angiitis Obliterans, Arch. Int. Med. **55**:942 (June) 1935.

11. Dale, H. H.: On Some Physiological Actions of Ergot, J. Physiol. **34**: 163, 1906.

12. Herrick.[6] Pool and Nason.[7] Lewis and Gelfand.[8] Heymans, C.; Bouckaert, J. J., and Moraes, A.: Au sujet de l'action vasculaire de l'ergotamine: inversion de l'action vaso-constrictrice des "vasotonines" du sang défibriné par l'ergotamine, Compt. rend. Soc. de biol. **110**:993, 1932.

13. Pool, J. L.; von Storch, T. J. C., and Lennox, W. G.: Effect of Ergotamine Tartrate on Pressure of Cerebrospinal Fluid and Blood During Migraine Headache, Arch. Int. Med. **57**:32 (Jan.) 1936.

delenburg [14] was able to produce relaxation of the smooth muscle of the bronchi, and Wiechmann [15] demonstrated a similar effect on the smooth musculature of isolated strips of stomach and intestine. Teschendorf,[16] using strips of the musculature of the skin of the leech, which is a pure smooth muscle, was also able to relax the intense spasm induced by barium chloride and prevent contraction from faradic stimulation by adding magnesium chloride to the bathing fluid. As has been mentioned previously, we have been able to prevent spasm of the smooth muscle of the passively sensitized guinea-pig both in vivo and in vitro with the magnesium ion.[1] These studies on preparations of isolated smooth muscle furnish strong evidence that magnesium acts directly on the smooth muscle fiber. However, Wiechmann pointed out that he was able to demonstrate a paralyzing action on the myoneural junction in addition to the direct paralyzing action on the muscle fiber.

From this evidence it is our belief that magnesium relieved the hypertension in the rats which were given ergotamine by a peripheral relaxing action on the smooth muscle coats of the blood vessels. There was no evidence that the narcotic action of magnesium played any rôle in effecting this reduction in blood pressure. The rats fed on the magnesium diet were always active. Demonstrable dehydration was not produced in the animals on the magnesium diet and therefore cannot be considered as the mechanism for reducing the blood pressure.

As has been pointed out, in rats on both diets which were given ergotamine gangrene of the tail developed. It is felt that the failure of the magnesium ion to prevent this change in the tail was due to the fact that the vasoconstriction immediately following the injection of the large doses of ergotamine tartrate is so intense that the relaxing effect of magnesium when given by mouth is inadequate. This is further substantiated by our observation that the blood pressure is elevated in both groups of animals immediately after the injection of ergotamine and that the observed differences in pressure are apparent only after about one week. It is possible, however, that magnesium administered parenterally might be effectual in preventing the intense vascular spasm which is primarily responsibe for the gangrene.

In one group of rats, not included here, in which the blood pressure was maintained within normal limits with the magnesium diet after three

14. Trendelenburg, P.: Physiologische und pharmakologische Untersuchungen an der isolierten Bronchialmuskulatur, Arch. f. exper. Path. u. Pharmakol. **69**: 79, 1912.

15. Wiechmann, E.: Zur Theorie der Magnesium Narkose, Arch. f. d. ges. Physiol. **182**:74, 1920.

16. Teschendorf, W.: Beiträge zur Physiologie und Pharmakologie der Blutegelmuskulatur, Arch. f. d. ges. Physiol. **192**:135, 1921.

injections of ergotamine, after the injection of three additional doses of ergotamine tartrate the blood pressure was no longer controlled by the magnesium diet in some of the animals.

The clinical implications of this study are obvious. They suggest that the beneficial action of magnesium in lowering the hypertension in the acute phase of hemorrhagic nephritis may in a large measure be due to its relaxing effect on the musculature of the spastic vessels responsible for the elevation of the blood pressure. They further suggest that magnesium may be of therapeutic value in various vascular spastic states with or without hypertension.

SUMMARY

Hypertension was produced in rats by the parenteral injection of ergotamine tartrate.

Magnesium carbonate when added to the diet reduced the blood pressure of rats that had been rendered hypertensive to within normal limits.

The action of the magnesium ion in relieving this experimental hypertension is believed to be due to its relaxing effect on the smooth musculature of the blood vessels.

The clinical implications of this study are suggested.

Progress in Internal Medicine

LIVER AND BILIARY TRACT

A REVIEW FOR 1936

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The clinical literature dealing with diseases of the liver and the biliary tract continues to be voluminous. Much of it deals with case reports and clinical discussions; hence detailed summary is not necessary. We have sought, therefore, not to attempt a comprehensive review of this literature but to follow the plan used in previous reviews of this series [1] and to limit our discussion to selected subjects in which there have been noteworthy contributions. Even in these subjects we make reference to selected articles rather than give an inclusive bibliography.

PHYSIOLOGY OF THE BILIARY TRACT

Pressure Relationships Within the Biliary Tract.—It is generally accepted that the secretion of bile by the liver is continuous, but its discharge into the duodenum is intermittent. Both the discharge of bile into the duodenum and the filling and emptying of the gallbladder are controlled by the choledochal-duodenal sphincter—the sphincter of Oddi. Numerous observers have studied the pressure relationships within the biliary tract since the initial experiments of Doyon [2] and of Bainbridge and Dale.[3] These studies have been summarized by Ivy.[4]

From the Clinic for Diseases of the Liver and Biliary Tract of the Departments of Medicine and Surgery, the New York Post-Graduate Medical School and Hospital and the Long Island College Division of the Kings County Hospital.

1. Greene, C. H.; Bercovitz, Z., and Hanssen, E. C.: Liver and Biliary Tract: Review of the Literature for 1933 and 1934, Arch. Int. Med. **55**:681-706, (April) 1935.

2. Doyon, M.: Mouvements spontanés des voies biliares, Arch. de physiol. norm. et path. **5**:710-719, 1893.

3. Bainbridge, F. A., and Dale, H. H.: The Contractile Mechanism of the Gall-Bladder and Its Extrinsic Nervous Control, J. Physiol. **33**:138-155, 1905-1906.

4. Ivy, A. C.: The Physiology of the Gall Bladder, Physiol. Rev. **14**:1-102, 1934.

Most of the experiments were performed on anesthetized animals, but Higgins and Mann,[5] Potter and Mann,[6] McMaster and Elman,[7] and Ivy and Oldberg [8] so arranged their experiments that they were able to obtain readings on unanesthetized dogs.

They found that the sphincter of Oddi in the resting animal possesses a definite tonus and is able to withstand a pressure of from 90 to 250 mm. of water. This is greater than the pressure, 100 mm., in the resting gallbladder, so that in the fasting animal the secreted bile is stored in the gallbladder. After a meal the sphincter relaxes and the gallbladder contracts and empties. The maximal pressure developed in the gallbladder during contraction varies from 210 to 270 mm. of bile.

The maximal pressure under which bile is secreted by the liver, according to the reports of Naunyn,[9] Herring and Simpson,[10] Mitchell and Stifel,[11] Mann and Foster,[12] McMaster and Elman [7] and others, varies, in the dog, from 240 to 310 mm. of water. This pressure under ordinary circumstances is greater than the contractile force of either the gallbladder or the sphincter of Oddi, so that the liver normally is protected against the effects of back pressure. However, Ivy reported that spasm of the sphincter may occasionally produce a pressure of 800 mm. of water. Spasm of such intensity not only would prevent evacuation of the gallbladder but would cause biliary colic and, if it persisted, jaundice.

Less is known regarding the pressure relationships in the human biliary tract, though presumably they are similar to those in the dog. Robitschek and Turlot [13] in 1921 reported that the secretory pressure

5. Higgins, G. M., and Mann, F. C.: Observations on the Emptying of the Gall Bladder, Am. J. Physiol. **78**:339-348, 1926.

6. Potter, J. C., and Mann, F. C.: Pressure Changes in the Biliary Tract, Am. J. M. Sc. **171**:202-217, 1926.

7. McMaster, P. D., and Elman, R.: On the Expulsion of Bile by the Gall Bladder: Reciprocal Relationship with the Sphincter Activity, J. Exper. Med. **44**:173-198, 1926.

8. Ivy, A. C., and Oldberg, E.: Hormone Mechanism for Gall Bladder Contraction and Evacuation, Am. J. Physiol. **86**:599-613, 1928.

9. Naunyn, B.: Zur Naturgeschichte der Gallensteine und zur Cholelithiasis; Mitt. a. d. Grenzgeb. d. Med. u. Chir. **14**:537-554, 1905.

10. Herring, P. T., and Simpson, S.: The Pressure of Bile Secretion and the Mechanism of Bile Absorption in Obstruction of the Bile Duct, Proc. Roy. Soc., London, s.B **79**:517-532, 1907.

11. Mitchell, W. T., and Stifel, R. E.: The Pressure of Bile Secretion During Chronic Obstruction of the Common Bile Duct, Bull. Johns Hopkins Hosp. **27**:78-79, 1916.

12. Mann, F. C., and Foster, J. P.: Secretory Pressure of the Liver, Am. J. Physiol. **47**:278-282, 1918.

13. Robitschek, W., and Turlot, M.: Der Sekretionsdruck der Galle beim Menschen, Wien. klin. Wchnschr. **34**:263 (June 2) 1921.

of the bile varied in human patients from 210 to 270 mm. Koster, Shapiro and Lerner [14] reported one case in which they found the normal tonus of the sphincter of Oddi to equal 230 mm. of water. McGowan, Butsch and Walters [15] were interested in changes in the pressure in the common bile duct, and in their experiments they did not attempt to determine the total pressure. However, they reported spasm of the sphincter of Oddi with an intensity equivalent to a pressure of over 600 mm. of water. The pressure exerted by contraction of the human gallbladder has not been reported.

Cholangiography.—The direct roentgenographic visualization of the biliary tree by the injection of iodized poppy-seed oil or some other roentgenopaque solution into a biliary fistula or into a drainage tube anchored in the gallbladder or bile ducts was introduced into clinical medicine by Lanari and Squirri [16] in 1924. Immediate cholangiography or the visualization of the gallbladder or bile ducts at the time of operation was advocated by Mirizzi.[17] These methods have been extended and popularized by Ginzburg and Benjamin,[18] Walters and Thiessen,[19] Ross,[20] Hicken, Best and Hunt,[21] Robins and Hermanson,[22] and others. It is becoming generally accepted that cholangiography is a valuable diagnostic procedure in that it enables the presence of calculi, strictures or neoplasms or the course of a fistula affecting the biliary passages to be demonstrated visually and gives direct evidence

14. Koster, H.; Shapiro, A., and Lerner, H.: On the Rate of Secretion of Bile, Am. J. Physiol. **115**:23-26, 1936.

15. McGowan, J. M.; Butsch, W. L., and Walters, W.: Pressure in the Common Bile Duct of Man: Its Relation to Pain Following Cholecystectomy, J. A. M. A. **106**:2227-2230 (June 27) 1936.

16. Lanari, E. L., and Squirri, C. M.: Roentgenography of Biliary Passages After Lipiodol Injections, Rev. Asoc. méd. argent. **37**:125-127, 1924.

17. Mirizzi, P. L.: La cholangiografía durante las operaciones de las vías biliares, Progresos de la clín. **41**:213-221, 1933.

18. Ginzburg, L., and Benjamin, E. W.: Lipiodol Studies of Post-Operative Biliary Fistulae, Ann. Surg. **91**:233-241, 1930.

19. Walters, W., and Thiessen, N. W.: Visual Methods of Studying the Physiology of the Common Bile Duct: I. The Problem of Pancreatitis and Sphincteritis, Proc. Staff Meet., Mayo Clin. **9**:772-775 (Dec. 19) 1934.

20. Ross, J. C.: The Use of Lipiodol in the Surgery of the Biliary Passages, Lancet **1**:251-254 (Feb. 1) 1936.

21. Hicken, N. F.; Best, R. R., and Hunt, H. B.: Cholangiography: Visualization of the Gall Bladder and Bile Ducts During and After Operation, Ann. Surg. **103**:210-229, 1936. Best, R. R., and Hicken, N. F.: Cholangiographic Demonstration of Biliary Dyssynergia and Other Obstructive Lesions of the Gallbladder and Bile Ducts, J. A. M. A. **107**:1615-1619 (Nov. 14) 1936.

22. Robins, S. A., and Hermanson, L.: Cholangiography: Modified Technic for X-Ray Visualization of Bile Ducts During Operation, Surg., Gynec. & Obst. **62**:684-688, 1936.

regarding the patency of the biliary passages. In many patients, regardless of the presence of dilatation, infection of the bile ducts or calculi, the injected iodized oil passes into the duodenum without delay. In other cases there is retention of the oil in the biliary tree for periods up to three hours before it passes into the duodenum. This delay Best and Hicken,[23] and Hicken, Best and Hunt [21] ascribe to spasm or hypertonicity of the choledochal sphincter, and in a series of seventy-five cases they found evidence of such spasm in 15 per cent.

One of the roentgenograms published by Saralegui [24] as illustrative of spasm of the sphincter is particularly interesting, for it shows the common duct filled with contrast medium. The ampulla of Vater is also filled with contrast medium, which extends up into the pancreatic ducts. The opening of the papilla of Vater is closed, though there is contrast medium in the duodenum. There seems to be a separate sphincter between the ampulla of Vater and the common duct. This roentgenogram, therefore, seems to indicate that the choledochal-duodenal sphincter really consists of two parts, and so is a most interesting confirmation of the views of Westphal [25] and Lueth.[26]

The Clinical Syndromes Associated with Biliary Stasis.—The demonstration by objective methods of increased tone or spasm of the choledochal sphincter is a development of the last few years, but the recognition of its importance in clinical medicine has come through clinical observations over a prolonged period. Krukenberg [27] in 1903 described the occurrence of biliary colic in the absence of gallstones. The extensive literature dealing with these functional disturbances was summarized by Ivy and Sandblom [28] in 1934. Ivy and Sandblom discussed the physiologic basis for the concept of functional disturbance or biliary dyskinesia.

The clinical syndromes produced by such dyskinesia have been discussed by Greene, Twiss and Carter [29] under the heading of biliary stasis. They pointed out that at least three types of disturbance produc-

23. Best, R. R., and Hicken, N. F.: Biliary Dyssynergia: Physiological Obstruction of the Common Bile Duct, Surg., Gynec. & Obst. **61**:721-734, 1935.

24. Saralegui, J. A.: Die Cholangiographien bei Studien der Gallenleiden [fig. 6], Fortschr. a. d. Geb. d. Röntgenstrahlen **52**:571-579, 1935.

25. Westphal, K.: Muskelfunktion, Nervensystem und Pathologie der Gallenwege: I. Untersuchungen über den Schmerzanfall der Gallenwege und seine ausstrahlenden Reflexe, Ztschr. f. klin. Med. **96**:22-150, 1923.

26. Lueth, H. C.: Studies on Flow of Bile into the Duodenum and Existence of Sphincter of Oddi, Am. J. Physiol. **99**:237-252, 1931.

27. Krukenberg, H.: Ueber Gallenblasenkoliken ohne Gallenstein, Berl. klin. Wchnschr. **40**:667, 1903.

28. Ivy, A. C., and Sandblom, P.: Biliary Dyskinesia, Ann. Int. Med. **8**: 115-122, 1934.

29. Greene, C. H.; Twiss, J. R., and Carter, R. F.: Biliary Stasis, Am. J. Digest. Dis. & Nutrition **3**:622-624, 1936.

tive of stasis and an associated symptomatology can be recognized clinically. These types are of importance both in diagnosis and in treatment.

1. Obesity and a loss of muscle tone are observed in many women as the menopause is approached. Numerous observers have emphasized the increasing proportion of cases of achlorhydria that are observed in older persons. The presence of acid chyme in the duodenum not only causes relaxation of the sphincter of Oddi but stimulates evacuation of the gallbladder. Its absence in achlorhydria has the opposite effect, resulting in distention of the gallbladder. This may be referred to as atonic distention. The resultant biliary stasis is signaled clinically by epigastric or hypochondriac soreness or distress, which is most marked during fasting. Colic is infrequent. Achlorhydria or hypochlorhydria is shown by analysis of gastric contents. Gastro-intestinal roentgenograms usually show nothing abnormal. In the cholecystogram the shadow of the gallbladder appears distended and atonic. It may show delay in either filling or emptying. Duodenal drainage may procure a large quantity of concentrated bile, but frequently this is secured only after stimulation with olive oil. The biliary stasis is further shown by the presence of crystalline sediment. In such cases the condition responds to therapeutic management with a stimulating diet and the administration of hydrochloric acid, and a fatty diet or of olive oil between meals to stimulate evacuation of the gallbladder.

2. It has long been known that in many instances of duodenal ulcer the symptoms are those of disturbance of the gallbladder. In a further number of cases roentgen study of the gastro-intestinal tract shows duodenitis, periduodenal adhesions, pylorospasm, gastric ulcer and the like in association with disease of the gallbladder. In such cases it can be assumed that the increased motility and tone of the duodenal musculature and the sphincter of Oddi interfere with the normal evacuation of the gallbladder. Because there is an increased resistance to its emptying, the gallbladder becomes distended and hypertonic. This may be referred to as a hypertonic or spastic distention of the gallbladder. Clinically it is characterized by frequent attacks of colic, which may be accompanied by jaundice. Gastric hyperacidity usually is present. Roentgenograms of the gastro-intestinal tract show evidences of pylorospasm and spasm, hypermotility or increased tonus of the duodenal musculature. Cholecystograms show the gallbladder to be distended and hypertonic. Duodenal drainage shows evidence of stasis in the frequency in which concentrated bile is procured only after stimulation with olive oil. The bile is dark and concentrated, and the presence of crystalline sediment is further evidence of the stasis. In such cases the condition responds to therapeutic management with diets of the modified

ulcer type, antispasmodic drugs, such as belladonna, and alkalis to control the hyperacidity.

3. The association of symptoms of disease of the gallbladder with neuroses of various types is no new observation. The association of chronic appendicitis, a spastic colon, pelvic disease or pregnancy with exacerbations of the symptoms of biliary stasis has been explained on a reflex basis. In such cases it may be assumed that there is a spasm of the sphincter of Oddi as a result of vagal stimulation which may arise directly in the central nervous system or reflexly in some other portion of the abdominal cavity. This spasm or increased tonus of the sphincter of Oddi interferes with the normal evacuation of the gallbladder which then becomes distended. This may be referred to as a neurogenic or vagotonic distention of the gallbladder. The resultant biliary stasis is characterized clinically by the presence of psychogenic factors or sources of reflex irritation which may explain the vagotonia. Biliary colic and even jaundice may occur. The gastric acidity is usually within the normal range, and gastro-intestinal roentgenograms do not show evidences of local lesions in the stomach or duodenum. Cholecystograms show the gallbladder to be distended, and hypertonic duodenal drainage shows evidence of stasis in the concentration of the bile and the presence of crystalline sediment. Concentrated bile, however, is usually obtained after stimulation with a solution of magnesium sulfate. In such cases the condition responds to therapeutic management with change of environment, mental rest, sedatives, antispasmodics and saline cathartics. It is this group in particular that is responsible for the vogue of "spa" therapy in the treatment of chronic cholecystitis.

The evaluation of the exact rôle of the individual factors of stasis, infection and metabolic changes in the production of disease of the gallbladder is difficult, but the concept of biliary stasis explains many of the clinical features of this disease. Failure to correct the stasis is responsible for many instances of recurrence of symptoms following cholecystectomy. Greene, Twiss and Carter pointed out that biliary stasis is not a single disease entity but may be the result of any one of various types of disturbance in the motor activity of the gallbladder and the biliary tract. They expressed the belief that these three main types at least can be recognized and that the recognition of them permits both a more accurate diagnosis and a more specific and satisfactory therapeutic management of the individual patient.

Pharmacologic Studies.—The effectiveness of nitrites, glyceryl trinitrate and related compounds in producing vasodilatation is well known. Their action, however, is not limited to the smooth muscle of the blood vessels. The pharmacologic studies of Lieb and McWhorter[30] showed

30. Lieb, C. C., and McWhorter, J. E.: Action of Drugs on the Isolated Gall-Bladder, J. Pharmacol. & Exper. Therap. **7**:83-98, 1915.

that they could produce relaxation of the gallbladder. Beams [31] stressed their value in clinical medicine in relieving cramps or spasm of the intestine. Their value in producing relaxation of the sphincter of Oddi was shown by McGowan, Butsch and Walters and by Best and Hicken. We can further testify as to the value of amyl nitrite or glyceryl trinitrate in relieving both biliary colic and pylorospasm.

McGowan, Butsch and Walters [15] reported studies on a series of cholecystectomized patients. They were interested in changes in pressure in response to drugs rather than in the absolute values, so that their results cannot be compared directly with the data quoted previously. However, they reported that a rise in the intraductal pressure was accompanied by pain. The administration of morphine regularly produced a rise in pressure, and this was accompanied by pain. The administration of amyl nitrite or of glyceryl trinitrate on the other hand rapidly brought the intraductal pressure down to zero, with relief of the pain. These changes are most readily explained by the production of spasm of the sphincter of Oddi by morphine and the relief of this spasm by nitrites.

These studies have been extended by McGowan, Butsch and Walters [31a] and by Doubilet and Colp.[31b] They confirm the production of spasm of the sphincter of Oddi by therapeutic doses of morphine and dilaudid. A lesser degree of spasm was produced by pantopon (a mixture of the hydrochlorides of the opium alkaloids) or codeine. Atropine or hyoscine caused a decrease in the tonus of the sphincter, but the spasm produced by morphine was relieved only by amyl nitrite or glyceryl trinitrate. A few experiments also suggested that theophylline ethylene diamine had a relaxing effect. Histamine, alcohol, ephedrine, epinephrine, papaverine, physostigmine, acetylcholine, ergotamine, calcium chloride, caffeine and phenobarbital in therapeutic doses had no distinct effect on the tonus of the sphincter.

The increased knowledge of the physiology of the biliary tract accumulated during the past few years gives promise of adding new remedies to the list of those now used in the management of the biliary disorders.

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31. Beams, A. J.: The Effect of Nitrites on Pain and on the Motility of the Gastro-Intestinal Tract: I. Clinical Study, Arch. Int. Med. **49**:270-275 (Feb.) 1932.

31a. McGowan, J. M.; Butsch, W. L., and Walters, W.: The Use of Glyceryl Trinitrate (Nitroglycerine) for the Control of Pain Following Cholecystectomy, Ann. Surg. **104**:1013-1018, 1936. Butsch, W. L.; McGowan, J. M., and Walters, W.: Clinical Studies on the Influence of Certain Drugs in Relation to Biliary Pain and to Variation in Intrabiliary Pressure, Surg., Gynec. & Obst. **63**:451-456, 1936.

31b. Doubilet, H., and Colp, R.: Resistance of the Sphincter of Oddi in the Human, Surg., Gynec. & Obst. **64**:622-633, 1937.

Ergotamine tartrate has been introduced into internal medicine as a pharmacologic antagonist to epinephrine and has been used in depressing overactivity of the sympathetic nervous system. Lennox and von Storch,[32] O'Sullivan [33] and several other investigators have found it of value in the relief of migraine. The pathogenesis of migraine is not known, though on an insecure basis it has been ascribed to hepatic disturbance. Similarly, the explanation of the action of ergotamine tartrate in relieving migraine is not clear. Ergotamine tartrate was also used successfully in treating the pruritus of jaundice by Lichtman,[34] Snell and Keyes [35] and others. The indiscriminate use of ergotamine tartrate is dangerous. Yater and Cahill [36] and Gould, Price and Ginsberg [37] each reported a case of bilateral gangrene of the feet following the use of large doses of ergotamine tartrate for the relief of pruritus in jaundice. Ergotamine may be of value in the treatment of the hypotonic type of biliary stasis, but it should be used with caution.

Choline and acetylcholine derivatives which stimulate the vagus or the parasympathetic nerve endings [38] likewise may be of value in the treatment of the hypotonic type of biliary stasis. The absorption of these two compounds after oral administration is variable, and they must be given hypodermically with caution because of the rapid but transitory fall of blood pressure which they produce. Acetylbetamethylcholine is now given by intranasal pack [39] or through the skin by ionto-

32. Lennox, W. G., and von Storch, T. J. C.: Experience with Ergotamine Tartrate in One Hundred and Twenty Patients with Migraine, J. A. M. A. **105**: 169-171 (July 20) 1935.

33. O'Sullivan, M. E.: Termination of One Thousand Attacks of Migraine with Ergotamine Tartrate, J. A. M. A. **107**:1208-1212 (Oct. 10) 1936.

34. Lichtman, S. S.: Therapeutic Response to Ergotamine Tartrate in Pruritus of Hepatic and Renal Origin, J. A. M. A. **97**:1463-1464, (Nov. 14) 1931.

35. Snell, A. M., and Keyes, H. C.: Pruritus of Jaundiced Patients: Its Incidence and Treatment, M. Clin. North America **16**:1455-1470, 1933.

36. Yater, W. M., and Cahill, J. A.: Bilateral Gangrene of Feet Due to Ergotamine Tartrate Used for Pruritus of Jaundice: Report of a Case Studied Arteriographically and Pathologically, J. A. M. A. **106**:1625-1631 (May 9) 1936.

37. Gould, S. E.; Price, A. E., and Ginsberg, H. I.: Gangrene and Death Following Ergotamine Tartrate (Gynergen) Therapy, J. A. M. A. **106**:1631-1635 (May 9) 1936.

38. Kovacs, J.; Saylor, L. L., and Wright, I. S.: The Pharmacological and Therapeutic Effects of Certain Choline Compounds, Am. Heart J. **11**:53-65, 1936. Myerson, A.; Rinkel, M., and Dameshek, W.: The Autonomic Pharmacology of the Gastric Juices, New England J. Med. **215**:1005-1013 (Nov. 26) 1936.

39. Van Dellen, T. R.; Bruger, M., and Wright, I. S.: The Absorption of Acetyl-B-Methylcholine Chloride (Mecholyl) by the Nasal Mucous Membrane, J. Pharmacol & Exper. Therap., to be published.

phoresis.[40] Further experience is necessary to determine the value of this group of drugs in diseases of the biliary tract.

Both ephedrine sulfate and benzedrine sulfate have been introduced into clinical medicine as sympatheticomimetic compounds which may be given orally.[41] Van Liere, Lough and Sleeth[42] studied the effect of 1 grain (0.065 Gm.) of ephedrine sulfate on the emptying time of the human stomach in six normal subjects. Gastric evacuation was prolonged in all. They expressed the opinion that ephedrine has two distinct actions on the stomach. First, it may stimulate the sympathetic fibers, which would cause diminished motility, and, second, it may actually relax the smooth muscle directly. Myerson and Ritvo[43] found that benzedrine sulfate in doses of 30 mg. by mouth is of great value in diminishing or abolishing spasm of the gastro-intestinal tract. Gastric tonus was reduced slightly, but peristaltic action was not interrupted. The stomach often emptied more rapidly after the administration of the drug because the relaxation of the pyloric sphincter allowed food to pass out of the stomach more rapidly than normally. These two compounds are similar in their actions; nevertheless, they show slight but important differences in their effects. It is possible that they will be of value in the treatment of spastic disturbances involving the biliary tract, but detailed studies have not been reported.

Effect of Cholecystectomy.—The experiments of Puestow[44] indicate that after cholecystectomy sphincteric tone is lost and bile dribbles into the duodenum as secreted. The effect of cholecystectomy in abolishing the normal tone of the sphincter would seem to be transitory, for Potter and Mann found the pressure in the common duct to be increased after cholecystectomy in dogs, and Judd and Mann[45] reported that in dogs the common bile duct uniformly becomes dilated after cholecystectomy. Puestow and Morrison[46] measured the circumference of the common

40. Kovacs, J.: The Iontophoresis of Acetyl-Beta-Methylcholine Chloride in the Treatment of Chronic Arthritis and Peripheral Vascular Disease: Preliminary Report, Am. J. M. Sc. **188**:32-36, 1934.

41. Myerson, A.; Loman, J., and Dameshek, W.: Physiologic Effects of Benzedrine and Its Relationship to Other Drugs Affecting the Autonomic Nervous System, Am. J. M. Sc. **192**:560, 1936.

42. Van Liere, E. J.; Lough, D. H., and Sleeth, C. K.: The Effect of Ephedrine on the Emptying Time of the Human Stomach, J. A. M. A. **106**:535-536 (Feb. 15) 1936.

43. Myerson, A., and Ritvo, M.: Benzedrine Sulfate and Its Value in Spasm of the Gastro-Intestinal Tract, J. A. M. A. **107**:24-26 (July 4) 1936.

44. Puestow, C. B.: The Discharge of Bile into the Duodenum: An Experimental Study, Arch. Surg. **23**:1013-1029 (Dec.) 1931.

45. Judd, E. S., and Mann, F. C.: The Effect of Removal of the Gall Bladder: An Experimental Study, Surg., Gynec. & Obst. **24**:437-442, 1917.

46. Puestow, C. B., and Morrison, R. B.: The Relationship of Cholecystitis and Cholecystectomy to Dilatation of the Choledochus, Ann. Surg. **101**:599-602, 1935.

duct in a series of patients and found that while the presence of chronic cholecystitis with or without stones had little effect on the size of the common duct, cholecystectomy was followed by a considerable degree of dilatation. The gallbladder by virtue of its storage function serves to protect the common duct from the effects of increased pressure within the biliary tract, and the loss of this protection undoubtedly explains the increase in the frequency and severity of biliary colic observed in some patients after cholecystectomy.

Carter [47] discussed the importance of a study of the response of the sphincter of Oddi in those cases in which surgical drainage of the common duct is necessary. This is a procedure which is generally advocated for stones, cholangeitis or chronic pancreatitis, but there has been no general agreement as to how long postoperative drainage is necessary. Ravdin stressed the importance of chemical and microscopic study of the bile as a guide to the persistence of infection and the severity of hepatic injury. Pribram,[48] Thorlakson and McMillan,[49] Walters and Thiessen and Hicken, Best and Hunt all pointed out that cholangiographic studies show that prolonged drainage of the common bile duct produces a reduction in the size of the dilated bile ducts, with a disappearance of the cholangiectasia. Carter admitted the importance of these different criteria of drainage but stressed the value of the food test. He measured the hourly drainage of bile during the day. When the sphincter of Oddi responds normally to food, the external drainage of bile is decreased or ceases entirely after meals. If the sphincter does not respond to food, the amount of biliary drainage is increased after the meal. A normal response to this food test indicates that the common duct is draining freely into the duodenum and the drainage tube in the common duct may safely be removed.

THE LIVER AND THE METABOLISM OF FAT

Fat Absorption.—The importance of the bile in the absorption of fats has long been known. This is due partly to its action in activating the pancreatic lipase, partly to the action of the bile salts in favoring emulsification and solution of fats and so hastening lipolytic action and partly to the formation of soluble addition compounds of bile salts and fats or fat-soluble materials. These addition compounds are absorbed directly into the blood stream. The recent investigations of

47. Carter, R. F.: When to Remove the Drainage Tube in Common Bile Duct Drainage, Surg., Gynec. & Obst. **63**:163-169, 1936.

48. Pribram, B. O.: New Methods in Gall Stone Surgery, Surg., Gynec. & Obst. **60**:55-64, 1935.

49. Thorlakson, P. H., and McMillan, J. C.: Common Duct Obstruction with Lipiodol Studies of Cholangiectasia Showing Effects of Prolonged Drainage, Canad. M. A. J. **31**:265-269, 1934.

Verzár [50] and of Drummond [51] emphasizing the importance of bile in the absorption both of neutral fats and of fat-soluble materials were reviewed in 1936 [52] and need not be discussed in detail at the present time.

The rate of absorption of fats is often mentioned in clinical literature as a valuable aid in the appraisal of the normal functioning of the liver. Sullivan and Fershtand [53] have studied the estimation of the degree of alimentary lipemia as an index of the rate of absorption of fat. Curves for absorption of fat by normal men and by patients with disease of the liver were obtained by following the changes in total fats of the serum subsequent to the ingestion of 100 Gm. of cottonseed oil. Patients with definite disease of the liver showed a curve for absorption distinctly different from the normal and denoting a diminished and delayed absorption of ingested fat. This altered curve, based on values for fat in the blood, is deemed by the investigators sufficiently characteristic and different to be of aid in determining the functional status of the liver.

Although other reports support the use of this procedure in investigating the function of the liver, the results should be interpreted with caution. The quantity of fat in the blood at a given time is subject to the influence of a number of factors. The rate of uptake from the alimentary tract and the rate of deposition of the absorbed material in the cells of the body are important regulators of the level of blood fats. It should be remembered that lipemia occurs in association with diabetes as well as with starvation and does not, on the other hand, ordinarily develop in cases of chronic pancreatitis or carcinoma affecting other abdominal organs. These few instances of alteration of the blood fat levels unrelated directly to the functioning of the liver suffice to emphasize the limited importance that should be attached to absorption of fat, or alimentary lipemia, as a diagnostic measure of a hepatic condition.

Nachlas, Duff, Tidwell and Holt [54] attempted to avoid these difficulties by studying the lipemic curve after the intravenous administration

50. Verzár, F.: Absorption of Fats, Nutrition Abstr. & Rev. **2**:441-450, 1933. Verzár, F., and Laszt, L.: Studies on the Absorption of Fatty Acids, Biochem. Ztschr. **270**:24-35, 1934.

51. Drummond, J. C.: Biochemical Studies of Liver Function in Relation to Fat Metabolism, in Harvey Lectures, 1932-1933, Baltimore, Johns Hopkins Press, 1934, pp. 202-217.

52. Greene, C. H.: Liver and Biliary Tract: A Review of Certain Recent Contributions, Arch. Int. Med. **57**:1039-1054 (May) 1936.

53. Sullivan, M., and Fershtand, J. A. B.: Fat Adsorption: Its Value as an Index of Function of the Liver, Arch. Int. Med. **55**:834-841 (May) 1935.

54. Nachlas, A.; Duff, G. L.; Tidwell, H. C., and Holt, L. E.: Liver Function as Tested by the Lipemic Curve After Intravenous Fat Administration, J. Clin. Investigation **15**:143-151, 1936.

of highly emulsified fat. They noted a rise in the curve for blood fat to a level above normal after the administration of carbon tetrachloride to dogs. A similar rise was found in one case of congenital malformation of the bile ducts. In consequence, they expressed the belief that this test is worthy of further clinical study.

Large amounts of fat are present in the stools of patients with obstructive jaundice. Müller [55] and Munk,[56] who made the first quantitative studies of this phase of fat metabolism, reported that the amount of fat lost in the feces varies up to 78 per cent of that included in the diet. It is now recognized that considerable quantities of lipid materials are excreted into the bowel, and in normal persons will account for the greater part of the fecal lipids. The increased excretion of fats in the stool in obstructive jaundice may be due to the presence of unabsorbed fat, to an increased excretion by the bowel or to a combination of these factors. In order to determine the origin of the fat in the feces of subjects with bile fistulas, Shapiro, Koster, Rittenberg and Schoenheimer [57] fed fat containing a known proportion of deuterium. This isotope of hydrogen apparently behaves as ordinary hydrogen in metabolism and yet can be quantitatively determined in compounds containing it. It therefore serves as an identifying "mark" for substances of which it is a part. The proportion of deuterium in the fecal fat equated with that in the fat ingested should thus serve as a reliable index to the origin of the fecal fat. It was found that of the deuterium-containing fat given with the food only 30 and 35 per cent, respectively, appeared in the feces of the two subjects studied. It was concluded from these analyses that the remaining 65 to 70 per cent of the fatty acids in the diet was absorbed and that the greater part of the fecal fat in these patients originated from the fat secreted into the intestinal tract. The subjects received 0.7 and 2.1 Gm. of the deuterium-containing fat in the diet. It appears that such small amounts of fat can largely be absorbed even in the absence of bile. If all the food fat follows the course of the deuterium-containing fat, fecal lipid in the absence of bile is largely endogenous. If these conclusions can be confirmed, present day views regarding the degree of interference with absorption of fat produced by the absence of bile from the intestines should be modified.

The Intermediary Metabolism of Fats.—After absorption from the intestine the fats are stored for the most part in the fat depots of the

55. Müller, F.: Untersuchungen über den Icterus, Ztschr. f. klin. Med. **12**: 45-113, 1887.

56. Munk, J.: Ueber die Resorption von Fetten usw. nach Abschluss der Galle vom Darmkanal, Virchows Arch. f. path. Anat. **122**:302, 1890.

57. Shapiro, Arthur; Koster, Harry; Rittenberg, D., and Schoenheimer, R.: The Origin of Fecal Fat in the Absence of Bile, Studied with Deuterium as an Indicator, Am. J. Physiol. **117**:525-529, 1936.

subcutaneous omental and retroperitoneal tissues. The fat is later withdrawn from these stores. The formation of unsaturated fatty acids from the saturated fats of the body has been looked on as one of the initial stages in the process of oxidation.

The studies of Burr and Burr [58] and of Evans and Lepovsky,[59] however, have shown that unsaturated fatty acids, such as linoleic and linoleinic acid, are necessary for the growth of rats. Ellis and Isbell,[60] Ellis and Zeller [61] and Banks and Hilditch [62] likewise agreed that linoleic acid in the body is entirely exogenous in origin. This observation that linoleic and linoleinic acids must be supplied in the diet is important if it can be confirmed in other species of animals besides the rat and pig, for it seems to indicate that the body is unable to desaturate fatty acids. Otherwise, these acids could readily be formed by the desaturation of oleic acid.

In the study of this problem, as in the study of many phases of intermediary metabolism, questions arise in which it would be desirable to distinguish between those molecules of a substance which come from the food and are exogenous and those which are formed in the body and are endogenous. Schoenheimer and Rittenberg [63] have attempted to mark molecules of fatty acid by the use of deuterium and so to follow the behavior of these marked molecules in the body. Unsaturated fats were saturated by hydrogenation with deuterium. The marked saturated fats were then fed to mice. At the completion of the experiment the animals were killed and the unsaturated fatty acids in the carcass were isolated. These unsaturated fatty acids contained an unduly high proportion of deuterium, showing that some were formed by the desatu-

58. Burr, G. O., and Burr, M. M.: On the Nature and Rôle of the Fatty Acids Essential in Nutrition, J. Biol. Chem. **86**:587-621, 1930.

59. Evans, H. M., and Lepkovsky, S.: Vital Need of the Body for Certain Unsaturated Fatty Acids: I. Experiments with Fat-Free Diets in Which Sucrose Furnishes the Sole Source of Energy, J. Biol. Chem. **96**:143-156, 1932; Vital Need of the Body for Certain Unsaturated Fatty Acids: II. Experiments with High Fat Diets in Which Saturated Fatty Acids Furnish the Sole Source of Energy, ibid. **96**:157-164, 1932.

60. Ellis, N. R., and Isbell, H. S.: Soft Pork Studies: III. The Effect of Food Fat upon Body Fat, as Shown by the Separation of the Individual Fatty Acids of the Body Fat, J. Biol. Chem. **69**:239-248, 1926.

61. Ellis, N. R., and Zeller, J. H.: Soft Pork Studies: IV. The Influence of a Ration Low in Fat upon the Composition of Body Fat of Hogs, J.Biol.Chem. **89**:185-197, 1930.

62. Banks, A., and Hilditch, T. P.: The Body Fats of the Pig: II. Some Aspects of the Formation of Animal Depot Fats Suggested by the Composition of Their Glycerides and Fatty Acids, Biochem. J. **26**:298-309, 1932.

63. Schoenheimer, R., and Rittenberg, D.: Deuterium as an Indicator in the Study of Intermediary Metabolism: V. The Desaturation of Fatty Acids in the Organism, J. Biol. Chem. **113**:505-510, 1936.

ration of the marked fat which had been fed. When unsaturated fatty acids labeled with deuterium were fed, the deuterium was later found in the saturated fatty acids obtained from the carcass.[64] These experiments therefore confirm in unmistakable fashion the older views that both the saturation and the unsaturation of fats take place in the body.

The older view of Leathes and Raper [65] insisted that the desaturation of fatty acids occurs in the liver. This theory was based on the observation that the fats in the liver usually are more unsaturated than those in the fat depots.

Various other explanations for this observation have been advanced, for the metabolism of unsaturated fats is not understood at the present time. Holt, Tidwell and Kirk [66] and Artom [67] found that they were better absorbed from the intestine than were saturated fats. Joannovics and Pick [68] showed that unsaturated fatty acids were selectively absorbed by the liver, and Sinclair [69] has shown recently that the liver not only selectively absorbs unsaturated fatty acids when cod liver oil is fed but retains them for a prolonged period.

The preferential absorption and retention by the liver of other fat-soluble materials, such as vitamin A, and some sterols, such as phytol, has been discussed by Drummond [51] and by Davies and Moore.[70] Unsaturated fatty acids may be absorbed from the blood stream in the same fashion. The significance of these observations unfortunately is not yet clear, and further study of the rôle of the liver in the metabolism of fats is desirable.

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64. Rittenberg, D., and Schoenheimer, R.: Deuterium as an Indicator in the Study of Intermediary Metabolism: VIII. Hydrogenation of Fatty Acids in the Animal Organism, J. Biol. Chem. **117**:485-490, 1937.

65. Leathes, J. B., and Raper, H. S.: The Fats, New York, Longmans, Green & Co., 1925.

66. Holt, L. E.; Tidwell, H. C., and Kirk, C. M.: Studies on Fat Metabolism in Infants, Acta pædiat. **16**:165-176, 1933.

67. Artom, C.: Fat Metabolism, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Stanford University Press, 1935, vol. 4, p. 199.

68. Joannovics, G., and Pick, E. P.: Experimentelle Untersuchungen über die Bedeutung der Leber bei der Fettresorption unter normalen und pathologischen Verhältnissen, Wien. klin. Wchnschr. **23**:573, 1910.

69. Sinclair, R. G.: Physiology of the Phospholipids, Physiol. Rev. **14**:351-403, 1934; The Metabolism of the Phospholipids: V. The Relationship Between the Amount of Fat Ingested and the Degree of Unsaturation of the Phospholipids and Neutral Fat in the Tissues of the Rat, J. Biol. Chem. **96**:103-125, 1932; The Metabolism of the Phospholipids: IV. The Rate of Phospholipid Metabolism with Special Reference to the Question of the Intermediary Rôle of the Phospholipids in Fat Metabolism, ibid. **95**:393-408, 1932.

70. Davies, A. W., and Moore, T.: Vitamin A and Carotene: Elimination of Vitamin A from Livers of Rats Previously Given Massive Doses of Vitamin A Concentrate, Biochem. J. **29**:147-150, 1935.

It has been generally accepted that fats are burned by the process of β oxidation. Combustion normally is complete, but in various pathologic conditions associated with a deficient supply of carbohydrate oxidation is incomplete and ketosis results. The older work emphasizing the importance of the liver in the formation of ketone bodies has been summarized by Fischler [71] and Snapper.[72] Confirmatory evidence has been introduced by Chaikoff and Soskin,[73] who showed that the ketone bodies in the blood of a depancreatized dog will fall rapidly after hepatectomy. Mirsky [74] confirmed the experiments of Anselmino and Hoffmann [75] showing that ketosis is produced in normal animals by the injection of the ketogenic principle of the anterior lobe of the hypophysis but further reported that ketosis did not follow the injection of this material in eviscerated rabbits. Himwich, Goldfarb and Weller [76] analyzed the arterial blood and the venous blood from various organs and found the liver to be the most constant source of ketone bodies.

The experiments of Jowett and Quastel,[77] Edson,[78] Trowell [79] and others also have shown that fatty acids are oxidized to aceto-acetic acid in vitro by slices of liver. More aceto-acetic acid was obtained by the oxidation of fatty acids with even-numbered carbon chains than by the

71. Fischler, F.: Physiologie und Pathologie der Leber nach ihrem heutigen Stande, ed. 2, Berlin, Julius Springer, 1925.

72. Snapper, I.: Ueber den intermediären Stoffwechsel der Niere, Verhandl. d. Gesellsch. f. Verdauungs. -u. Stoffwechselkr. **5**:92, 1925.

73. Chaikoff, I. L., and Soskin, S.: Utilization of Acetoacetic Acid by Normal and Diabetic Dogs Before and After Evisceration, Am. J. Physiol. **87**:58-72, 1929.

74. Mirsky, I. A.: The Source of Blood Acetone Resulting from the Administration of the Ketogenic Principle of the Anterior Hypophysis, Am. J. Physiol. **115**:424-428, 1936; Site and Mechanism of the Antiketogenic Action of Insulin, ibid. **116**:322-326, 1936.

75. Anselmino, K. J., and Hoffmann, F.: Das Fettstoffwechselhormon des Hypophysenvorderlappens: Nachweis, Darstellung und Eigenschaften des Hormons, Klin.Wchnschr. **10**:2380-2383, 1931.

76. Himwich, H. E.; Goldfarb, W., and Weller, A.: The Effect of Various Organs on the Acetone Content of the Blood in Phlorhizin and Pancreatic Diabetes, J. Biol. Chem. **93**:337-342, 1931.

77. Jowett, M., and Quastel, J. H.: Studies in Fat Metabolism: Oxidation of Butyric, Crotonic and B-Hydroxybutyric Acids in Presence of Guinea-Pig Liver Slices, Biochem. J. **29**:2143-2158, 1935; Studies in Fat Metabolism: Oxidation of Normal Saturated Fatty Acids in Presence of Liver Slices, ibid. **29**:2159-2180, 1935; Studies in Fat Metabolism: Formation and Breakdown of Aceto-acetic Acid in Animal Tissues, ibid. **29**:2181-2191, 1935.

78. Edson, N. L.: Ketogenesis-Antiketogenesis: Influence of Ammonium Chloride on Ketone-Body Formation in Liver, Biochem. J. **29**:2082-2094, 1935.

79. Trowell, O. A.: Choline and Liver Respiration, J. Physiol. **85**:356-374 (Nov. 22) 1935.

oxidation of those with an odd-numbered chain. This observation supports the old conception of β oxidation in the oxidation of fats, but in addition the possibility of there being other mechanisms whereby fat is oxidized must be kept in mind, for Quastel [80] found evidence for the oxidation of several other carbon atoms (multiple alternate oxidation). Verkade and van der Lee [81] also found evidence for an additional ω oxidation.

These experiments stress the importance of the liver in the oxidation of fat. It must not be concluded, however, that this is necessarily a function of the liver exclusively, for McMaster and Drury [82] showed that fat may be metabolized after hepatectomy.

Fatty Infiltration of the Liver.—The amount of fat which can be visualized by histologic methods in an organ such as the liver does not necessarily correspond with the amount that can be determined by chemical analysis. This difference has been explained by the existence in protoplasm of lipoprotein complexes in which the fat does not take up the usual stains. The pathologist therefore is interested in fatty degeneration as evidence of cellular injury or destruction. The clinician, on the other hand, considers the liver the chief chemical factory of the body and is interested in the problem of fatty infiltration as evidence of the transport of fat to the liver as one step in its metabolism. The relationship between these two conditions has not yet been determined with certainty, but progress in the understanding of the rôle of the liver in the metabolism of fat is being made.

The clinical picture of fatty liver occurs in a great variety of conditions. In many of these, as in diabetic coma, the triad of Geelmuyden [83]—mobilization of fat often with lipemia, fatty liver and ketonuria—is readily recognizable.

The clinical picture, however, is most variable, and many factors must be considered. Even in diabetes, enlargement of the liver with infiltration of fat may occur without associated ketonuria, as in the

80. Quastel, J. H.: Oxidation of Fatty Acids in the Liver, Arch. f. exper. Zellforsch. **15**:57, 1934.

81. Verkade, P. E., and van der Lee, J.: Researches on Fat Metabolism, Biochem. J. **28**:31-40, 1934; Untersuchungen über den Fettstoffwechsel, Ztschr. f. physiol. Chem. **225**:230-244, 1934; Untersuchungen über den Fettstoffwechsel: Zweiseitige β-Oxydation der durch ω-Oxydation gesättigter Fettsäuren enstandenen Dicarbonsäuren, ibid. **227**:213-222, 1934.

82. McMaster, P. D., and Drury, D. R.: The Relation of the Liver to Fat Metabolism: I. Respiratory Quotient in Conditions of Liver Insufficiency, Proc. Soc. Exper. Biol. & Med. **25**:151-153, 1927.

83. Geelmuyden, H. C.: Ueber Fettwanderung, Acta med. Scandinav. **54**:5, 1920.

cases of Hanssen.[84] Root [85] likewise reported four cases of diabetic coma with fatty liver. Lipemia was observed in three, but in all four acute pancreatitis was present. Bloom and Handelsman [86] reported a marked degree of fatty infiltration of the liver in a dog, with naturally occurring diabetes.

Fatty infiltration of the liver can be produced in experimental animals under a variety of circumstances. Fisher [87] and Allan, Bowie, McLeod and Robinson [88] noted that in depancreatized dogs treated with insulin in adequate dosage there nevertheless developed marked fatty infiltration of the liver, which eventually resulted in the death of the animal. The addition of raw pancreas to the diet prevented the development of the fatty liver. McLeod likewise noted that this fatty infiltration of the liver could be prevented by the feeding of a fat-free extract of the pancreas. Hershey and Soskin [89] found that lecithin can be substituted for the raw pancreas, though Ralli, Flaum and Banta [90] considered pancreas more effective than lecithin. Finally, a study of the component parts of the lecithin molecule by Best, Ferguson and Hershey [91] showed that the administration of choline would prevent the fatty infiltration of the liver in depancreatized dogs.

These observations stimulated an extensive series of investigations dealing with fatty infiltration. Feeding white rats a diet high in fat Best and Huntsman [92] were able to produce fatty infiltration of the liver. Diets rich in sugar and low in fat did not have this effect. Best and Ridout [93] found that diets rich in cholesterol caused fatty liver,

84. Hanssen, P.: Enlargement of the Liver in Diabetes Mellitus, J. A. M. A. **106**:914-916 (March 14) 1936.

85. Root, H. F.: Diabetic Coma and Acute Pancreatitis with Fatty Livers, J. A. M. A. **108**:777-780 (March 6) 1937.

86. Bloom, F., and Handelsman, M. B.: Diabetes in Dogs, North American Vet. **18**:39 (April) 1937.

87. Fisher, N. F.: Attempts to Maintain the Life of Totally Pancreatectomized Dogs, Am. J. Physiol. **67**:634-643, 1924.

88. Allan, F. N.; Bowie, J. J.; McLeod, J. J. R., and Robinson, W. L.: Behaviour of Depancreatized Dogs Kept Alive with Insulin, Brit. J. Exper. Path. **5**:75-83, 1924-1925.

89. Hershey, J. M., and Soskin, S.: Substitution of "Lecithin" for Raw Pancreas in the Diet of the Depancreatized Dog, Am. J. Physiol. **98**:74-85, 1931.

90. Ralli, E.; Flaum, G., and Banta, R. J.: Results of Feeding Lecithin and Pancreas in Depancreatized Dogs on Liver Fat and Its Saponifiable and Unsaponifiable Fractions, Am. J. Physiol. **110**:545-551, 1935.

91. Best, C. H.; Ferguson, G. C., and Hershey, J. M.: Choline and Liver Fat in Diabetic Dogs, J.Physiol. **79**:94-102, 1933.

92. Best, C. H., and Huntsman, M. E.: The Effects of the Components of Lecithin upon Deposition of Fat in the Liver, J. Physiol. **75**:405-412, 1932.

93. Best, C. H., and Ridout, J. H.: Choline and Fatty Liver, J.Physiol. **84**: 7P, 1935.

and Blatherwick, Medlar, Bradshaw, Post and Sawyer [94] obtained similar results by feeding liver itself.

The fatty deposits in the liver produced by a diet with a high fat content consists chiefly of neutral fats. According to Best, Channon and Ridout,[95] diets with a high cholesterol content produce an increase in both neutral fat and cholesterol, with a decrease in the phosphatide fraction. Channon and Wilkinson [96] found that the fat content of the liver increases more readily after the feeding of saturated fats than when unsaturated fats are fed. Kaplan and Chaikoff [97] also found that the fatty infiltration of the liver in depancreatized dogs is due to an increase in the neutral fats and to a lesser extent in cholesterol, with little or no change in the phosphatides.

In various papers Best [98] and his associates reported that choline reduces or prevents the fatty infiltration of the liver when high fat diets are fed. In reviewing the problem, Best stated that the administration of choline does not alter the rate of absorption of fat, nor does it affect the amount or character of the deposited fat, nor is there evidence at present that it increases the oxidation of fat in the liver. Best and Ridout showed that choline rapidly decreases the amount of neutral fat in the liver but only slowly reduces the accumulated cholesterol esters. Beeston, Channon and Loach [98a] found that the addition of casein to a diet with a high fat content supplemented the effect of choline in preventing fatty infiltration.

Other methods of producing fatty infiltration of the liver have been studied. The fatty liver produced by starvation is well known. Dible and Libman [99] showed that the degree of infiltration is influenced by the nutritive state of the animal and that most marked changes do not occur unless large deposits of fat are present. Best and Ridout found

94. Blatherwick, N. R.; Medlar, E. M.; Bradshaw, P. J.; Post, A. L., and Sawyer, S. D.: Occurrence of Fatty Livers in Rats Fed Diets Containing Liver, J. Biol. Chem. **97**:xxxiii, 1932.

95. Best, C. H.; Channon, H. J., and Ridout, J. H.: Choline and Dietary Production of Fatty Livers, J. Physiol. **81**:409-421 (July 31) 1934.

96. Channon, H. J., and Wilkinson, H.: The Effect of Various Fats in the Production of Dietary Fatty Livers, Biochem. J. **30**:1033-1039, 1936.

97. Kaplan, A., and Chaikoff, I. L.: Liver Lipids in Completely Depancreatized Dogs Maintained with Insulin, J. Biol. Chem. **108**:201-216, 1935.

98. Best, C. H.: The Rôle of the Liver in the Metabolism of Carbohydrate and Fat: III. The Deposition of Liver Fat, Lancet **1**:1274-1277 (June 16) 1934.

98a. Beeston, A. W.; Channon, H. J.; Loach, J. V., and Wilkinson, H.: Further Observations of the Effect of Dietary Caseinogen in Prevention of Fatty Livers, Biochem. J. **30**:1040-1046, 1936.

99. Dible, J. H., and Libman, J.: Further Observations on Fat Mobilization in Starvation, J. Path. & Bact. **38**:269-284, 1934.

that the effect of starvation on the liver was abolished by feeding choline for some weeks prior to the starvation period but that choline given during the period of starvation did not prevent fatty infiltration. Choline likewise diminished the amount of fat deposited in the liver after phosphorus poisoning, but according to Best, McLean and Ridout,[100] it did not reduce the toxicity of the phosphorus.

The posterior lobe of the pituitary gland influences fat metabolism, and this subject has been reviewed in detail by Raab.[101] Mukerji and van Dyke[102] reported that the fatty infiltration of the liver which follows a single injection of pitressin is not prevented by the administration of choline.

A new aspect of the aforementioned work has arisen with studies by Van Prohaska, Dragstedt and Harms[103] on the fatty infiltration of the livers of depancreatized dogs. They show that the changes in the liver were not due to the loss of the external pancreatic secretion, though Berg and Zucker[104] previously had reported that damage to the liver followed the production of a pancreatic fistula. Von Prohaska and his associates[105] demonstrated further that the small amount of raw pancreas needed to prevent the changes in the liver contained such a small amount of choline or lecithin that these constituents could not have been responsible for its effect in preventing fatty infiltration. Dragstedt, Van Prohaska and Harms later isolated a fat-free alcohol-soluble substance from the pancreas, which they called the "lipocaic hormone." When fed to dogs this material prevented the liver from becoming infiltrated with fat. These authors found that depancreatized dogs still required the substance after hypophysectomy despite improvement in the dietary status. This new work brings up the necessity of further study of the action of choline in its relation to the pancreatic hormone.

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100. Best, C. H.; McLean, D. L., and Ridout, J. H.: Choline and Liver Fat in Phosphorus Poisoning, J. Physiol. **83**:275-284 (Feb. 9) 1935.

101. Raab, W.: The Rôle of the Pituitary Posterior Hormone in Fat Metabolism, Endocrinology **14**:385-388, 1930.

102. Mukerji, B., and van Dyke, H. B.: The Effect of the Pressor Principle of the Posterior Lobe of the Pituitary Body on the Liver-Fat After the Feeding of Choline Chloride, Chinese J. Physiol. **9**:69-76, 1935.

103. Dragstedt, L. R.; Van Prohaska, J., and Harms, H. P.: Observations on a Substance in Pancreas Which Permits Survival and Prevents Liver Changes in Depancreatized Dogs, Am. J. Physiol. **117**:175-181, 1936.

104. Berg, B. N., and Zucker, T. F.: Liver Changes After Deprivation of External Pancreatic Secretion, Proc. Soc. Exper. Biol. & Med. **29**:68-70, 1931.

105. Van Prohaska, J.; Dragstedt, L., and Harms, H. P.: The Relation of Pancreatic Juice to the Fatty Infiltration and Degeneration of the Liver in the Depancreatized Dog, Am. J. Physiol. **117**:166-174, 1936.

Kaplan and Chaikoff [106] have studied the size and composition of the liver of a normal dog and of dogs under a variety of abnormal conditions, including depancreatized, hypophysectomized, hypophysectomized-depancreatized, phlorhizin-treated and thyroid-fed animals. The composition of the liver varied widely in these different conditions. However, the absolute amount of water and protein in the liver was relatively constant and was not affected by changes in the amount of either glycogen or fat.

These results suggest that the absolute amount of the active functioning protoplasm of the liver does not change in such acute experiments and that the changes in fat or glycogen represent the addition or subtraction of reserves which only indirectly affect the activity of the living protoplasm. The experiments of Addis, Poo and Lew [107] supplement these studies by indicating that so far as protein is concerned the liver may also be a storage depot for use in time of need.

This point of view is particularly important, since a great many previous experiments have been interpreted as showing not only that the deposition of glycogen in the liver is associated with the retention of considerable amounts of water but that there is also a considerable antagonism between the deposition of glycogen and that of fat. Nagao,[108] for example, found that the intravenous injection of fat produces a deposition of fat associated with disappearance of glycogen from the liver.

It has been recognized previously that depancreatized dogs show an increase in dextrose-tolerance when fatty infiltration of the liver occurs. Best, Huntsman and Young,[109] on the other hand, insisted that the apparent increase in the sugar tolerance in these dogs antedates the deposition in fat in the liver and that instead of there being an antagonistic relationship between the glycogen and fat of the liver, the derangement of both phases of metabolism is due to the same cause, a disturbed hepatic function which affects first the metabolism of sugar and later the metabolism of fat. These questions are of direct clinical importance with regard to both the causation and the significance of fatty infiltration of the liver, and it is hoped that definitive experiments which answer some of these questions conclusively will soon be reported.

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106. Kaplan, A., and Chaikoff, I. L.: The Relation of Glycogen, Fat, and Protein to Water Storage in the Liver, J. Biol. Chem. **116**:663-682, 1936.

107. Addis, T.; Poo, L. J., and Lew, W.: Protein Loss from Liver During a Two Day Fast, J. Biol. Chem. **115**:117-118, 1936.

108. Nagao, S.: The Penetration of Glycogen and Fat into the Parenchyma as Antagonistic Processes, Tohoku J. Exper. Med. **24**:555-564, 1934.

109. Best, C. H.; Huntsman, M. E., and Young, F. G.: The Effect of Diets Low in Choline, and of Choline Feeding, on the Glycosuria of the Depancreatized Dog, J. Physiol. **85**:8P, 1935.

THE METABOLISM OF AMINO-ACIDS AND AMMONIA IN DISEASE OF THE LIVER

The importance of the liver in protein metabolism has been repeatedly and conclusively demonstrated. Kirk [110] has recently reported the study of the metabolism of amino-acids and ammonia in a series of fifty-seven cases of hepatic disease. As part of the study, he discussed the analytic difficulties in the determination of these materials and furnished good reasons for disregarding most of the published data bearing on this problem. He found that the aminonitrogen in the plasma varies over the same range in patients with disease of the liver as in normal persons. Curves for tolerance obtained by administering 25 Gm. of amino-acetic acid likewise fell within the normal range. Analysis of urine both after fasting and after administration of 25 Gm. of amino-acetic acid showed that the excretion of aminonitrogen was the same in normal persons as in patients with hepatic disease. He therefore concluded that in his experiments an impairment of the mechanism for deaminizing aminoacids could not be demonstrated. The clinical value of tolerance tests with amino-acetic acid was questioned accordingly.

The concentration of ammonia in the blood normally is low, varying from 0.013 to 0.050 mg. per hundred cubic centimeters. Normal values were obtained in hepatic disease, with the exception of cirrhosis. In cirrhosis, on the other hand, the fasting concentration was increased, and it increased even more remarkably after the administration of ammonium citrate. Kirk reported additional evidence indicating that the ability to synthesize urea from ammonia was unimpaired. The concentration of ammonia in the blood of the portal vein is much greater than that in the peripheral blood stream, but the ammonia normally is almost completely removed by the liver. In persons with hepatic cirrhosis, who have a well developed collateral circulation, enough portal blood is shunted around the liver to affect the concentration of ammonia in the peripheral blood stream. Kirk therefore concluded that the determination of the ammonia in the blood in cirrhosis affords no information regarding the functional state of the liver but does furnish a method for studying the development of collateral circulation in such a case.

SYPHILIS OF THE LIVER

The many and varied types of hepatic lesion that have been ascribed to syphilitic infection were reviewed by O'Leary, Greene and Rowntree [111] in 1929. The gumma is the one hepatic lesion that is universally

110. Kirk, E.: Amino Acid and Ammonia Metabolism in Liver Diseases, Copenhagen, Levin & Munksgaard, 1936.

111. O'Leary, P. A.; Greene, C. H., and Rowntree, L. G.: Diseases of the Liver: VIII. The Various Types of Syphilis of the Liver with Reference to Tests for Hepatic Function, Arch. Int. Med. **44**:155-193 (Aug.) 1929.

accepted as being syphilitic in origin, but it seems probable that syphilitic disturbances of the liver are far more frequent than would be indicated by the incidence of hepatic gumma.

Biskind, Epstein, and Kerr [112] in 1934 reported a study of the bengal rose test for hepatic function in a series of one hundred and fifty-two syphilitic persons. After excluding all those in whom there was clinical evidence of hepatic involvement, they reported that functional disturbance could be demonstrated in over 17 per cent. Similar results were reported in 1936 by Kellogg, Epstein and Kerr.[113] These results are most important if they can be confirmed by other methods, for they indicate a frequency of hepatic injury far higher than is usually accepted.

A detailed survey of the literature, with especial reference to the factors responsible for jaundice in syphilis, was reported by Sager [114] with particular reference to the rôle of arsphenamine. He reported that transient slight jaundice is occasionally found in syphilitic cirrhosis or in gumma of the liver, but the clinical syndrome of acute yellow atrophy is not found in association with tertiary syphilis.

Acute jaundice associated with the secondary stage of syphilis, the icterus syphiliticus praecox, was the only type recognized in the prearsphenamine era. Sager suggested that this type of jaundice in untreated persons with secondary syphilis is caused by parenchymatous hepatic injury produced by the toxemia associated with systemic invasion by the spirochete. It apparently is not due to an inflammatory response of the liver to the presence of the spirochete.

Jaundice has been observed in syphilitic persons with increasing frequency since the introduction of arsphenamine. This increase has been noted both in secondary and in tertiary syphilis. Experimentally, arsphenamine damages the liver, and jaundice may be produced in dogs by large doses. The incidence of jaundice increases with the drug used. Jaundice also occurs after the therapeutic use of arsphenamine in nonsyphilitic persons. Other reactions caused by arsphenamine, such as arsphenamine dermatitis, occasionally appear with the jaundice. Sager therefore concluded that arsphenamine is the toxic agent responsible for the development of paratherapeutic or postarsphenamine jaundice

112. Biskind, G. R.; Epstein, N. N., and Kerr, W. J.: Hepatic Complications in the Treatment of Syphilis: The Rose Bengal Test as a Means of Detecting Disturbances of Liver Function and Its Use as a Guide in the Therapy of Syphilis, Ann. Int. Med. **7**:966-980, 1934.

113. Kellogg, F.; Epstein, N. N., and Kerr, W. J.: Hepatic Complications in the Treatment of Syphilis: II. Incidence of Hepatic Disease in Patients with Untreated Syphilis and During Their Subsequent Treatment, Ann. Int. Med. **9**:1561-1571, 1936.

114. Sager, R. V.: Factors Responsible for Jaundice in Syphilis with Special Reference to the Rôle of the Arsphenamines, Arch. Int. Med. **57**:666-694 (April) 1936.

and that this type of jaundice is a separate etiologic variety distinct from the usual varieties of catarrhal jaundice.

THE TREATMENT OF HEPATIC DISEASE

The treatment of hepatic disease is neither fully understood nor appreciated by the medical profession as a whole. Treatment in general can be divided into four main divisions: (1) general care of the patient apart from the disease of the liver, (2) the treatment of parenchymal injury, (3) the treatment of ascites and (4) symptomatic treatment for the relief of specific symptoms.

General Care.—The general care of the patient apart from the disease of the liver is an individual question and need not be reviewed at this time.

The Treatment of Parenchymal Injury.—1. Dextrose: The agents which may damage the liver are varied in the extreme, and no attempt will be made to catalog them. There are ample experimental data as well as corroborative clinical evidence that a supply of glycogen is essential to the normal functioning of the liver. When adequate reserves of glycogen are present, not only is the liver more resistant to injury but regeneration and the restoration of normal function are more rapid than when these reserves are depleted. The glycogen supply of the liver is normally built up from the carbohydrate in the food.[115] Such experiments as those of Tsai,[116] Butsch[117] Salter, Robb and Scharles[118] show that glycogen is formed equally well from dextrose given by vein and from that administered orally. London,[119] Tsai and Yi,[120] Olmsted and Reed[121] and Giragossintz and Olmsted,[122] on the

115. Bollman, J. L., and Mann, F. C.: Experimentally Produced Lesions of the Liver, Ann. Int. Med. **5**:699-712, 1931. Ivy, A. C. and Crandall, L. A.: Liver and Bile, in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Stanford University Press, 1936, vol. 5, pp. 427-447.

116. Tsai, C.: Carbohydrate Metabolism of Liver: Glycogen and Other Carbohydrates in Decapitate Cats, Chinese J. Physiol. **7**:215-226 (Dec. 15) 1933.

117. Butsch, W. L.: Glucose Tolerance and the Glycogen Storage Capacity of the Dog, Am. J. Physiol. **108**:639-642, 1934.

118. Salter, W. T.; Robb, P. D., and Scharles, F. H.: Liver Glycogen from Derivatives of Glucose, J. Nutrition **9**:11-23, 1935.

119. London, E. S.: Agiostomie und Organstoffwechsel, Moscow, Des All-Union-Instituts für exper. Med., 1935.

120. Tsai, C., and Yi, C. L.: Carbohydrate Metabolism of Liver: Sugar Output in Amytalized Cats, Chinese J. Physiol. **8**:399-408 (Nov. 15) 1934.

121. Olmsted, J. M. D., and Reed, L. S.: Glucose and Non-Glucose Portions of "Blood Sugar" in the Hepatic and Portal Veins of the Decapitate Cat at Different Sugar Levels, Am. J. Physiol. **109**:303-306, 1934.

122. Giragossintz, G., and Olmsted, J. M. D.: Portal and Hepatic Blood Sugar After Glucose Administration, Proc. Soc. Exper. Biol. & Med. **32**:668-670, 1935.

other hand, found that the dextrose content of the blood from the hepatic vein is greater than that of blood from the portal vein even at times when glycogen is being deposited in the liver. These results seem to indicate that glycogenolysis by the liver and the liberation of dextrose into the hepatic veins are a continuous process. These investigators suggest that the hepatic glycogen may not be formed directly from the dextrose in the blood but from some other glycogenic substance.

Experience has demonstrated that an ample intake of carbohydrate ordinarily will result in the deposition of glycogen in the liver. A high carbohydrate diet therefore is indicated in the treatment of acute injury of the liver and is usually used in jaundice. When the patient is able to eat, there is no reason why the carbohydrate should not be administered orally in a high carbohydrate diet. The general rule in such cases,[122a] as exemplified by the reports of Althausen [123] and Jones,[124] is to supply between 300 and 500 Gm. of carbohydrate. When the patient cannot eat such a diet, other methods of supplying carbohydrate must be sought. When weakness, anorexia or coma make feeding unsatisfactory, tube feeding should be tried. When a duodenal tube can be put in place, the administration of liquids in a continuous drip is most satisfactory.

Enemas of dextrose have not been satisfactory in our hands, for after the initial trial they are poorly retained and cause much discomfort.

A 5 per cent solution of dextrose may be given by hypodermoclysis, but the total amount which may be given in this way is small, and unless the patient is badly dehydrated the dextrose is accompanied by an unduly large volume of water. The intravenous infusion of a solution of dextrose in concentrations of from 10 to 25 per cent has come to be the method of choice for supplying it to the patient with acute disease of the liver.

The continuous intravenous infusion of a solution of dextrose as reported by Hendon [125] and others is occasionally of value. Technical difficulties, particularly with regard to preventing thrombosis or occlusion of the vein, however, militate against its use except under special conditions.

It has been suggested that whenever a solution of dextrose is administered intravenously insulin should be given to insure the utiliza-

122a. Martin, L.: Jaundice: Methods of Diagnosis and Treatment of Its Causes, Bull. Johns Hopkins Hosp. **59**:78-98, 1936.

123. Althausen, T. L.: Dextrose Therapy in Diseases of the Liver, J. A. M. A. **100**:1163-1167 (April 15) 1933.

124. Jones, C. M.: The Treatment of Acute Hepatic Insufficiency and Its Relation to Prognosis, Am. J. Digest. Dis. & Nutrition **3**:624-629, 1936.

125. Hendon, G. A.: Experiences with Venoclysis, Ann. Surg. **91**:753-760, 1930.

tion of the dextrose. Our experience has indicated that except in diabetic patients little dextrose is excreted in the urine after its intravenous administration. This loss is reduced but not abolished by the simultaneous injection of insulin, and it may be concluded that the insulinogenic function of the pancreas is unimpaired in the great majority of patients with hepatic disease. In fact, Althausen warns that the continuous intravenous injection of dextrose should be decreased gradually and not stopped abruptly, because of the danger of insulin shock in consequence of the stimulation of production of insulin by the dextrose.

2. Protein in the Diet: Meat and meat extracts should be eliminated from the diet of all patients with severe hepatic disease. The danger of meat intoxication in dogs with an Eck fistula was emphasized by Fishler. Bollman and Mann pointed out that dogs on a high meat diet showed more extensive necrosis of the liver after carbon tetrachloride poisoning than did dogs on a high carbohydrate diet. Furthermore, they pointed out that in dogs with severe hepatic injury the development of ascites can be precipitated by the administration of meat or meat extracts.

The protein intake should not be restricted too rigidly. One of us (C. H. G.[82]) reviewed the evidence indicating that the liver is active in the formation of the serum protein and pointed out that evidences of a reduction in the serum protein or a change in the albumin-globulin ratio or both was found in many patient's with hepatic disease. The majority of papers have stressed the toxicity of meat rather than that of protein. We therefore doubt the advisability of reducing the protein in the diet below 1 Gm. per kilogram of body weight, especially if that protein is given in the form of eggs and milk or dairy products.

The palatability of the diet is important, especially when it is necessary for the patient to follow a prescribed regimen for a prolonged period. Meat is most valuable in affecting the palatability of the diet, and we believe that many patients with cirrhosis follow their diet better if small amounts of meat are allowed occasionally.

3. Fat in the Diet: If large amounts of carbohydrate are given in the diet, the fat will automatically be reduced. Many physicians believe that a fat-free diet is essential. However, butter and cream and egg yolks usually are well tolerated by the patient with hepatic disease and need not be eliminated from the diet, even though they do supply a modicum of fat.

Hepatic injury or jaundice induced by arsphenamine represents a special group. Craven[126] has shown that when dogs kept on a high

126. Craven, E. B.: The Importance of Diet in Preventing Acute Yellow Atrophy During Arsphenamine Treatment, Bull. Johns Hopkins Hosp. **48**:131-142, 1931.

fat diet are given arsphenamine there is less hepatic necrosis than in dogs on a high carbohydrate diet. It is to be hoped that this question will be reinvestigated in detail and the applicability of these studies to patients be proved. Cross and Blackford [127] have shown the development of severe hypoglycemia in subacute yellow atrophy of the liver secondary to administration of arsphenamine. The indications for the selection of a high fat or a high carbohydrate diet in such cases are not clear at the present time.

4. Calcium Therapy: The experiments of Minot and of Cutler [128] have shown that the hepatic necrosis of carbon tetrachloride poisoning in dogs may be diminished by the administration of both calcium and dextrose. Part of the benefit observed may be due to the action of calcium in neutralizing the effect of guanidine and related compounds, which are toxic and part to a hastening of glycogenolysis.

Most interesting are the recent experiments of Forbes, Neale and Scherer [129] dealing with the isolation of a compound of commercial liver extracts which protects the liver from the toxic effects of carbon tetrachloride.

The Treatment of Ascites.—The medical management of ascites is primarily a problem of the effective use of diuretics, a topic which has been thoroughly reviewed by Hayman [130] and by Keith.[131] They pointed out that the diuretics of greatest value in the treatment of ascites are mercurial compounds, acid-producing or potassium salts and purine diuretics.

The Addison pill of ancient lineage, containing digitalis, squill and mercury, is still a popular diuretic for the treatment of ascites, but the greatest usefulness is found in organic compounds of mercury, such as merbaphen, salyrgan and mercurin (the sodium salt of trimethylcyclopentane-dicarboxylic acid-methoxymercuryhydroxideallylamide).

Numerous organic mercurial diuretics have been reported, but accumulated clinical experience indicates that of the group described salyrgan and mercurin have the greatest diuretic effect and produce minimal toxic symptoms. The usual practice is to give 0.5 cc. intra-

127. Cross, J. B., and Blackford, L. M.: Fatal Hepatogenic Hypoglycemia Following Neoarsphenamine, J. A. M. A. **94**:1739-1742 (May 31) 1930.

128. Cutler, J. T.: The Influence of Diet on Carbon Tetrachloride Intoxication in Dogs, J. Pharmacol. & Exper. Therap. **45**:209-226, 1932.

129. Forbes, J. C.; Neale, R. C., and Scherer, J. H.: A Liver Preparation Protecting Against Necrosis from Chloroform or Carbon Tetrachloride Administration, J. Pharmacol. & Exper. Therap. **58**:402-408, 1936.

130. Hayman, J. M.: The Clinical Use of Diuretics, J. A. M. A. **107**:1937-1941 (Dec. 12) 1936.

131. Keith, N. M.: The Action and Use of Diuretics with Especial Reference to Mercurial Compounds, J. A. M. A. **107**:2047-2051 (Dec. 19) 1936.

venously as an initial trial dose. If there are no toxic effects, 2 cc. is given every three or four days as long as there is a satisfactory diuretic response. The mercurial compounds are irritating, and care must be used in making the injection, else there is danger of subcutaneous necrosis. They may be given intramuscularly, but this method of administration is usually painful and not as satisfactory as the intravenous route. Recently both mercurin and salyrgan have been supplied in suppositories for rectal administration containing 0.5 Gm. of the mercurial salt. Parkinson and Thomson,[132] Fulton,[133] Flexner [134] and others have reported satisfactory diuretic response to the use of such suppositories, especially in cases of cardiac edema.

Mercurial diuretics are contraindicated or should be used with extreme caution in acute nephritis or in the presence of renal insufficiency with incipient uremia. When used with discretion and care in properly selected cases, they may be given over long periods without any evidence of renal injury. However, the possibility of renal injury should not be forgotten, and the physician must be on the lookout for evidences of its appearance. This danger is not eliminated by the introduction of the mercurial suppository, for one of us (C. H. G.) has observed a case in which temporary anuria resulted from the administration of a mercurin suppository.

The various inorganic acid-producing salts have a moderate diuretic action of their own, but they show an even more striking effect in augmenting the diuretic action of the organic mercurial compounds. The most effective acid salts have been ammonium chloride and ammonium nitrate. Keith has also stressed the value of potassium nitrate. Large doses of these various compounds are required, the recommended daily dose being ammonium chloride 9 Gm., ammonium nitrate 12 Gm. and potassium nitrate 12 Gm. They all are irritant to the stomach and may produce vomiting; so they are best administered at meal time in 0.5 Gm. enteric-coated pills. The most marked acidifying effect of ammonium chloride is of relatively short duration, being greatest on the second and third days of administration. It is therefore best given intermittently—as on the first three days of the week. If the mercurial compound is given less frequently, the acid salt should be administered for two days before and throughout the day the mercurial compound is injected.

The purine or xanthine diuretics, theophylline, theobromine and caffeine or their derivatives, usually are effective in the order named. They are of less importance in the treatment of ascites due to portal

132. Parkinson, J., and Thomson, W. A. R.: A Mercurial (Nouvurit) Suppository as a Diuretic for Cardiac Edema, Lancet **1**:16-19 (Jan. 4) 1936.

133. Fulton, M. N.: Mercurin Suppositories as a Diuretic in the Treatment of Edema, New England J. Med. **214**:1092-1095 (May 28) 1936.

134. Flexner, J.: Personal communication to the authors.

cirrhosis than they are in cardiac dropsy. However, there is some evidence that they enhance the effectiveness of the mercurial compounds.

Hayman also reported that urea may be an effective diuretic in ascites.

It is generally agreed that the most dramatic response to the therapeutic use of diuretic drugs occurs in cases of congestive heart failure. They are effective in many cases of early portal cirrhosis, but the more advanced the hepatic insufficiency the less the response to diuresis. In our experience, some patients respond well to a diuretic regimen but as time and presumably the severity of the lesion progress the response lessens and finally disappears. The ascites occasionally noted in acute hepatitis likewise responds poorly to treatment with diuretics.

Many investigators have suggested that the liver is active in water metabolism, and the aforementioned observations suggest that diuresis is less easily produced, as the functional efficiency of the liver is reduced. This view is also supported by the observations of Jones and Eaton [135] regarding the good prognostic significance of spontaneous diuresis in hepatic disease. Most patients with cirrhosis with ascites should be on a salt-poor diet, and the fluid intake should be moderately restricted, say to 1,000 to 1,200 cc. The advantages of more rigid restriction are usually counterbalanced by the unpalatability of the required diet and the consequent loss of appetite.

Purgation formerly was much used in the management of ascites. The modern view is to relegate this type of therapy to the discard, for the benefit is not sufficient to counterbalance the many and obvious disadvantages.

Paracentesis remains the measure of last resort in the management of ascites. Frequent tapping should be avoided if possible, for much protein is lost in the ascitic fluid, and this loss not only increases the nutritional disturbance but hastens the depletion of the plasma proteins, a circumstance which carried to an extreme will produce peripheral edema and, in lesser degree, undoubtedly favor the production of ascites. The patient with a distended, fluid-filled abdomen on the other hand is much more comfortable and subjectively feels much better if the excess of fluid is removed by paracentesis and diuretics are used to prevent or delay its reaccumulation than if an attempt is made to treat the condition solely by medical measures.

Symptomatic Treatment for the Relief of Specific Symptoms.—A multitude of individual symptoms that require care and attention on the part of the physician are observed in cases of hepatic disease. Only a few of the more important ones will be referred to here.

135. Jones, C. M., and Eaton, F. B.: The Prognostic Significance of a Spontaneous Diuresis in Acute or Subacute Disease of the Liver, New England J. Med. **213**:907-918 (Nov. 7) 1935.

1. Anemia: The occurrence in cases of severe hepatic disease of macrocytic hyperchromic anemia with a blood picture resembling that seen in pernicious anemia was discussed previously. Further reports have been published during the year in confirmation of these observations. Shumacker and Wintrobe,[136] and Higgins and Stasney [137] also reported the experimental production of macrocytic anemia in dogs in which severe and long continued hepatic damage and cirrhosis had been produced by the administration of carbon tetrachloride. Fortunately this anemia ordinarily responds to the parenteral administration of liver extract, and such therapy is indicated in any case of hepatic disease in which there are evidences of an increase in the color index or a change in the volume of the erythrocytes.

2. Serum Proteins: The serum proteins frequently are reduced in portal cirrhosis. In most cases there is a deficiency in serum albumin, with a tendency toward an inversion of the albumin-globulin ratio.

The Takata-Ara test and similar tests produce positive reactions. Three factors contribute to the development of these changes: interference with the production of the serum proteins by the damaged liver; the depletion of serum proteins as a result of loss of protein through frequent tapping and the withdrawal of large amounts of ascitic fluid; a reduction in the protein intake as a result of either restriction of protein in the diet or anorexia or other digestive disturbance which prevents the intake or utilization of protein.

The reports of Foley, Keeton, Kendrick and Darling,[138] O'Hare and Driscoll,[139] and Myers and Keefer [140] emphasize these different factors but show that inanition or a deficient protein intake will produce a reduction in the plasma proteins without change in the albumin-globulin ratio. The reduction in the plasma proteins may be combated in part by a careful increase in the protein in the diet, though the response usually is disappointing. There usually is no direct relationship between the reduction in the plasma proteins and the loss in the ascitic fluid. These investigators agree that the degree of hypoproteinemia tends to

136. Shumacker, H. B., and Wintrobe, M. M.: Morphologic Changes in Blood Associated with Experimentally Produced Hepatic Damage, Bull. Johns Hopkins Hosp. **58**:343-377, 1936.

137. Higgins, G., and Stasney, J.: Peripheral Blood in Experimental Cirrhosis of Liver, Folia haemat. **54**:129-144, 1936.

138. Foley, E. F.; Keeton, R. W.; Kendrick, A. B., and Darling, D.: Alterations in Serum Proteins as an Index of Liver Failure, Proc. Soc. Exper. Biol. & Med. **33**:430-431, 1935.

139. O'Hare, J. P., and Driscoll, M.: Blood Proteins in Liver Disease, in Medical Papers Dedicated to Henry Asbury Christian, Physician and Teacher, in Honor of His Sixtieth Birthday, Baltimore, Waverly Press, Inc., 1936, p. 638.

140. Myers, W., and Keefer, C.: Relation of Plasma Proteins to Ascites and Edema in Cirrhosis of Liver, Arch. Int. Med. **55**:349-359 (March) 1935.

be proportional to the degree of cirrhosis and express the belief that it is the consequence of a diminished production of plasma proteins by the damaged liver. Treatment therefore is best directed toward combating the hepatic injury. In other cases, transfusion is indicated for its value in supplying both erythrocytes and plasma proteins.

3. Pruritus: Pruritus, which is one of the most disturbing symptoms of the patient with hepatic disease, has been discussed by Snell and Keyes. The patient with obstructive jaundice often has most dramatic relief after the reestablishment of the flow of bile. On the other hand, the patient with cirrhosis or chronic hepatitis who has pruritus presents a therapeutic problem of the first magnitude. Ergotamine tartrate, already referred to, may be of great benefit. The intravenous administration of sodium thiosulfate in 1 Gm. doses sometimes produces equally great relief, as may calcium gluconate administered intravenously. Small doses of histamine subcutaneously have been used by Ernstene and Banks [141] in the treatment of pruritus of urticarial origin. One of us (A. M. B.) has used it successfully for two patients with jaundice. Duodenal drainage or mild mercurous chloride at times seems to stimulate the flow of bile sufficiently to reduce the intensity of the itching. Diathermy may help. Local measures, such as bran or soda baths or lotions or salves containing phenol or local anesthetics, usually give but transient benefit. There seems to be no sure way of telling in advance which of these measures will relieve any individual patient. While the search is being made, the patient should be given sedatives in sufficiently large doses to secure rest and a modicum of comfort.

141. Ernstene, A. C., and Banks, B. M.: Use of Histamine in the Treatment of Pruritus: Preliminary Report, J. A. M. A. **100**:328-330 (Feb. 4) 1933.

Book Reviews

Collected Writings. By Alfred F. Hess. Price, $15. Pp. 1443, with 127 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1936.

To those who long knew and loved the man, who appreciated the fertility and the versatility of his mind, who were familiar with his work as it appeared and who looked forward eagerly to each new development, it is still a revelation to scan the 1,400 pages of the two volume "Collected Writings" of Alfred Hess. After a foreword by Mrs. Hess and a sympathetic biographic memoir by his friend Abraham Flexner in collaboration with E. A. Park, there are in chronological order nearly 200 papers, with 43 omitted "in order that the volumes should not be too cumbersome."

The work is a marvelous record of achievement dominated by a personality that was in many ways unique. It is no mere posthumous tribute when Park says that Hess was "the foremost investigator among pediatricians in this country. There was no one else who could possibly be compared with him. He was probably the foremost pediatric investigator in the world." And yet, paradoxically, as Flexner says in his memoir: "Hess was not really a master of any special method of investigation. He was not a bacteriologist, a chemist, a histologist or a physiologist. Without complete equipment, therefore, in any of the underlying sciences he was nevertheless one of the very foremost investigators of his time in medicine. One might add that he was not an outstanding clinician in the usual sense. As revealed in these collected writings, he combined and made all these special technics subservient to a method that was peculiarly his own. This has been admirably stated by Park: "Hess was a great skirmisher on the forefront of scientific progress, with an almost uncanny eye for the point at which the next attack should be made, with a genius for predicting the course which investigation should take, with almost infallible judgment on the basis of slight evidence and simple experiment as to where the weak point in the armor of ignorance lay. It was fortunate that a man with this scouting sense was not held back to command a regular body of troops. He blazed many paths, leaving other workers less daring and less imaginative to complete the work, while he passed on to other points of vantage."

This is evident in the chronological sequence of his work as shown in these volumes. A line of investigation was taken up and followed for a time and either seen to a conclusion or, after the way had been pointed, left to others to pursue into farther ramifications. As a result, one can roughly divide Hess' work into epochs. Beginning with a single case report and historical summary, he rapidly entered on a period of intensive interest in contagious diseases, notably tuberculosis. This was overlapped by the introduction of the duodenal catheter, with important demonstrations as to the pathogenesis of the hard casein curds in the stools of infants and the nature and flora of the pancreatic juice, and opened up wide avenues to further investigation. This subject was abruptly dropped because, as Hess characteristically told this reviewer, he "was no longer interested in going on with it—others could do that." There followed a short period of investigation into the nature of the disorders of the blood and of capillary resistance, leading to the demonstration, since confirmed by Aschoff and Wolbach, that the hemorrhages of infantile scurvy are due to the pathologic permeability of the capillaries. From that time on his great work, with a few deviations, dealt with deficiency diseases. First came a brilliant series of reports on the nature, pathogenesis and therapy of infantile scurvy. This again was all but terminated when his comprehensive book entitled "Scurvy, Past and Present" appeared in 1920. With the exception of some illuminating reports on vitamin A and dental caries, nearly all his later investigations centered on rickets, his outstanding single achievement. Discovery after discovery, not by chance but by the use of that

"almost uncanny eye for the point at which the next attack should be made," followed in rapid succession, among the most far reaching being the discovery that inert substances can be made incredibly rich in vitamin D by ultraviolet irradiation. Again there came a comprehensive summary in a book published in 1929 entitled "Rickets, Osteomalacia and Tetany," about which Finkelstein wrote him: "There has been no medical book in many years which has fascinated me so much and has given me so much pleasure and such edification in equal measure by its content and form." Much has been added to our knowledge of the vitamin D complex since that time, and the last 350 pages of the "Collected Writings" are an eloquent record of Hess' further contributions to that subject. His wise counsel and mature judgment, both leading and restraining, were never needed more, in this connection, than at the present time. It is a pity that Nature is at times so lavish and so ruthless. These volumes are a fitting memorial to the great and lovable personality—a stimulating reminder to future generations of students of medicine.

Synopsis of Clinical Laboratory Methods. By W. E. Bray, M.D. Price, $3.75. Pp. 324, with 32 illustrations and 11 color plates. St. Louis: C. V. Mosby Company, 1936.

This book consists of sixteen chapters which cover with few exceptions those clinical laboratory procedures that are simple and of proved clinical value.

The first chapter discusses in a general way the procedures that are part of the routine of the various hospital services or which may be needed on special indication. It gives a bird's-eye view of the equipment and tests necessary in each particular field.

The second chapter is a clear and complete presentation of the routine and special examinations that may be made of a specimen of urine. The Addis count, however, is omitted. The third chapter deals with hematology and is followed by chapters on blood chemistry, gastric analysis, feces analysis, intestinal parasites, examination of the cerebrospinal and other puncture fluids and examination of sputum. Throughout these chapters the methods of greatest simplicity have been critically selected, and any method which sacrifices reasonable accuracy to obtain simplicity has been deleted. Wherever possible the apparatus required is that which may be found in a laboratory not overly elaborate and expensively equipped.

The chapter on bacteriology is as concise and inclusive a fifty page treatise on the detection of pathogenic micro-organisms as one can conceive.

The chapter on water and milk analysis is only four pages long, and while necessarily incomplete for any one especially interested in this field, it probably gives sufficient information to make a satisfactory determination of the purity of these two liquids.

The chapter on serology is concerned entirely with the complement fixation, flocculation and precipitin tests for syphilis and is up to the uniformly high standard of the rest of the book. The Kolmer modification of the Wassermann test and the Kahn and Kline tests are recommended.

The chapters on basal metabolism and allergy tests are very short (two and three pages, respectively), and while they give considerable information in such a brief space, additional reading in these fields would be necessary to equip one to derive the best results from these procedures. The chapter on poisons and foreign substances is one that under ordinary circumstances will not be needed often but if needed at all will be badly needed. The detection of ordinary poisons is here clearly described.

The chapter on surgical pathology would of course require special training and much additional study and practice to be of much value to most physicians. Possibly it should have been omitted. The last chapter is composed of methods of preparation of over one hundred indicators, stains and reagents. Twenty-five random tests of the index proved it to be 100 per cent correct.

This book meets all the requirements of simplicity, clearness, accuracy and completeness that one could possibly lay down. It is written not for the research worker but for the medical student, for the laboratory technician and for the physician who is keeping abreast of the times and ever doing his best to practice modern medicine. It fulfills its purpose admirably and is hereby recommended to those who come within the scope of its declared purpose.

Glandular Physiology and Therapy: A Symposium Prepared Under the Auspices of the Council on Pharmacy and Chemistry of the American Medical Association. Cloth. Price, $2.50. Pp. 528. Chicago: American Medical Association, 1935.

There would be no great exaggeration in saying that all proved facts concerning glandular physiology and therapy are contained in this volume. The tone of this book is definitely conservative, and it is refreshing, indeed, to read a book on this subject in which the limitations and ignorance in the well populated field of endocrinology are so frankly admitted.

Scholarly papers set forth the results of well controlled laboratory work, and the success or failure of the clinical application of this work is ably discussed by competent clinicians.

It is surprising that it seemed necessary to devote nearly a fourth of the book to a discussion of the pituitary gland, but this was a wise procedure in view of the extensive amount of work recently accomplished in this field together with the seemingly unwarranted enthusiasm on the part of clinicians to tamper with the pituitary gland.

The gonads receive adequate attention, including an interesting but speculative chapter on carcinogenesis. A discussion of tests for pregnancy is included in this section.

The adrenal glands, thyroid and parathyroid glands, thymus and pancreas, including insulin therapy, are considered from a physiologic and clinical standpoint.

Gastro-intestinal principles are discussed, together with the antianemic material from the liver and stomach.

In the final chapter commercial glandular preparations are considered, and mention by name is made of commercial preparations that are thought to have value. The preparations are described together with the bases for their probable value. Mention is made also, but not by name, of preparations that are probably valueless. This chapter should be read with attention by every physician who prescribes glandular preparations.

It is more than likely that the enthusiastic but uncritical reader will consider this work far too conservative, but conservatism is needed today in endocrinology as well as in other fields.

Differentialdiagnose in der inneren Medizin. By O. Naegeli, M.D. Price, 9.60 marks. Pp. 216, with 61 illustrations. Leipzig: Georg Thieme, 1936.

This volume is the first of three which will represent the complete work of Naegeli on differential diagnosis in internal medicine. The second part is scheduled to appear in 1936 and the third in 1937, the total representing approximately 700 or 800 pages. This volume is short, containing 216 pages, and is paper bound.

Instead of approaching differential diagnosis from the standpoint of symptoms alone, Naegeli prefers to proceed from a broad point of view, first identifying the disease as an inflammation, intoxication, tumor, lymphatic or allied disorder, endocrine disturbance, allergic state, avitaminosis, deficiency disease or involvement of an isolated organ, such as the heart or brain. A brief discussion of each of these groups is given. For example, under inflammation the various criteria to establish such a diagnosis, such as the temperature, pulse rate, sedimentation time of the blood and leukocytic responses, are evaluated. The author then

plunges into a consideration of the differential diagnosis in specific groups. First, the anemias are considered and then polycythemia, the hemorrhagic diatheses, leukemias, disorders of the lymph nodes, mediastinal tumors, disturbances of the salivary glands, myelopathies, ulcerative lesions of the mouth, splenic disorders and disturbances of the liver and biliary tract.

Under each heading the important factors in differential diagnosis are stressed. The more important differentiations are taken up in relation to the symptom complex, such as isolated afebrile splenic tumor, enlargement of the lymph nodes and ulcerative lesions of the mouth. Frequent case reports illustrate the diagnostic procedure and problems at hand.

The work represents an approach to differential diagnosis that is seldom seen in English works. One is impressed by the absence of long lists of rare causes of usual symptoms and delighted to see an approach to differential diagnosis as it is practiced at the bedside. The book should find its place as a practical guide to the problems of differential diagnosis.

Food and Beverage Analyses. By Milton A. Bridges. Price, $3.50. Pp. 246. Philadelphia: Lea & Febiger, 1935.

Thirty years ago the investigations of Atwater and Bryant were published as Bulletin 28 of the United States Department of Agriculture, and this work has been a classic in the realm of nutrition, having been utilized all over the world. Since then practically nothing has been written of any moment concerning the analysis of foods, and this in spite of the fact that a host of new foods in various forms have been presented to the eating public.

Bridges has made an enormous number of original food analyses and in addition has had made an extensive search of international data in order to collect in one volume the information presented in this book. The result is an extremely complete summary of every known article of diet; at least any unusual article of food which the reviewer, picking at random, attempted to find absent was listed.

The arrangement of the material is such that the inquirer may refer to a given article with a minimum of trouble. The size of a portion of each article is given both in grams and in household measurements, and then the relative amount of carbohydrate, protein and fat, together with the total number of calories. The table of foods makes up the bulk of the book, but there are also sections devoted to the mineral content of food and to their vitamin value. One section deals with the composition and food values of alcoholic beverages, and one can find there just what is contained in a "Merry Widow" or a "Soul Kiss" cocktail as well as how to mix a slow gin fizz, make a mint julep or concoct a planter's punch.

This volume represents a tremendous amount of work; it presents to nutritionalist, dietitian and physician accurate information conveniently arranged. It is difficult to see how the physician can get along without it.

Basal Metabolism in Health and Disease. By Eugene F. Du Bois, M.D. Third edition. Cloth. Price, $5. Pp. 494, with 98 engravings. Philadelphia: Lea & Febiger, 1936.

Du Bois has correlated the investigations of pure science with the clinical problems of human metabolism. He begins with the related fundamentals of physics and biochemistry, creates an understanding of the interplay of forces defining and controlling normal metabolism and then discusses the pathologic physiology of metabolism in disease. These progressive steps are of great value to the student or to the practitioner whose knowledge of the fundamental sciences is limited, vague or out of date. No attempt is made to apply data difficult of interpretation to the gaps in one's knowledge of metabolism. Most of the information catalogued is acceptable when tested by present day methods and thinkers.

The third edition of this book contains recent information on surface area and normal standards and incorporates work done by J. D. Hardy, using a new type

of radiometer to study loss of heat. The discussion of undernutrition, obesity and allied states in diabetes provides the basis for intelligent dietary treatment of these conditions. The chapter on the thyroid gland is exceptionally well organized. The incomplete and contradictory state of the literature on the adrenal, pituitary and sex glands and their interrelationship has made metabolic studies of these conditions of little practical value. The chapters on diseases of the blood and on diseases of the heart and kidneys could be more adequately correlated with the pathologic physiology of these conditions. Water metabolism might be discussed in more detail. However, despite these few criticisms the volume may be heartily recommended. It presents authoritative information on metabolism in the language of the student and the clinician.

Endokrine Krankheiten. By Prof. Dr. Hans Curschmann, Direktor d. Medizin. Univ. Klinik i. M. With a contribution by Dr. med. et phil. Franz Prange, Nervenarzt in Rostock. **Medizinische Praxis, Band I.** Second Revised Edition. Price, unbound, 8 marks; bound, 9 marks. Pp. 144, with 47 illustrations. Dresden: Theodor Steinkopff, 1936.

This book is an abbreviation of the standard German texts on endocrinology and is especially rich in photographs of patients. The accounts are brief and accurate, but it is regrettable that in a book primarily for practitioners there should be inadequate discussion of certain elementary principles of therapy. The indications for surgical procedure and the proper preoperative use of iodine in cases of exophthalmic goiter are hardly touched on; the importance of sodium in adrenal insufficiency is not mentioned at all, and there is no adequate discussion of the essential chemical changes in hyperparathyroidism.

Grundriss der inneren Medizin. By Dr. A. von Domarus. Tenth edition. Price, 16.80 marks. Pp. 681, with 63 illustrations. Berlin: Julius Springer, 1936.

The number of editions through which this work has gone in its thirteen years of existence speaks not only for its popularity but for the keen interest of the author and publisher in keeping it up to date. Now in its tenth edition, it maintains a compactness and conciseness essential for student and quick reference use. True to the word *Grundriss* the discussions of the various disease states are brief. Tuberculosis, for example, is covered in eight pages and brucellosis in less than three. Essential facts, however, are not omitted, and discussions of controversial subjects are evaded. References are rarely given. The general plan and style of previous editions are maintained.

Die einheimische Sprue. By Dr. K. Hansen and Dr. H. von Staa. Price, 7.80 marks. Pp. 113, with 45 illustrations. Leipzig: Georg Thieme, 1936.

This excellent monograph, beautifully illustrated and with an extensive bibliography, covers the subject of endemic sprue. There are interesting reports of cases, and the question of the relation of this condition to idiopathic steatorrhea and osteomalacia is analyzed.